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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Solvent-free synthesis of novel (*E*)-2-(3,5-dimethyl-4-(aryldiazenyl)-1*H*-pyrazol-1-yl)-4-arylthiazoles: determination of their biological activity

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Abstract In search of novel biologically active azoles, some new (*E*)-2-(3,5-dimethyl-4-(aryldiazenyl)-1*H*-pyrazol-1-yl)-4-arylthiazoles **3a–p** have been synthesized via Hantzsch thiazole approach under greener, mild and solvent-free conditions. Structures of all compounds were established on the basis of the rigorous elemental analysis and their IR, NMR (^{1}H, ^{13}C), COSY, ROSEY, HSQC and HMBC spectral data. To explore the biological potential, all the synthesized compounds were evaluated for their antimicrobial, antioxidant and UV-mediated DNA damage-protective and photocleavage activity. The results of antimicrobial study revealed that among the series, compounds **3g–l** were found active selectively against *C. albicans*. The compound **3l** exhibited twofold high antifungal potential in comparison with amphotericin-B, a standard drug against *C. albicans*. The compound **3k** was found equipotent, and **3g–j** displayed half of the potential in reference to the standard drug. In UV-induced DNA damage-protecting and photocleavage study, **3j** and **3n** were found as the most potent DNA damage-protecting and DNA-photocleaving agents, respectively, at 40 μg concentration. The compounds **3d**, **3e**, **3g**, **3i**, **3k**, **3m** and **3o** also displayed a significant level of DNA damage-protective potential, whereas **3l** and **3p** exhibited appreciable DNA photocleavage activity. In the antioxidant study, all compounds were found inactive.

Keywords Antimicrobial · Antioxidant · DNA damage protective · DNA photocleavage · Arylazopyrazole · Pyrazolylthiazole

Introduction

Five-membered nitrogen-containing heterocycles are well known for their wide spectrum of therapeutic properties (Kumar *et al*., 2005, 2013a, b, 2014a, b; Kaur *et al*., 2014; Aggarwal *et al*., 2006). Among them, thiazoles, pyrazoles and thiazole–pyrazole hybrids have attracted great attention over the years due to their remarkable biological activities. In this context, the motifs containing thiazole nucleus exhibited antitrypanosomal (Zelisko *et al*., 2012), antimicrobial (Desai *et al*., 2013), anticancer (Romagnoli *et al*., 2012), anti-inflammatory (Helal *et al*., 2013) and antiviral (Barradas *et al*., 2011) properties. Some potent drugs like fanetizole (anti-inflammatory agent), tiazofurin (antineoplastic agent), penicillin (potent antibiotic), sulfatiazol (antimicrobial) and abafungin (antifungal) also possessed thiazole ring (Karthikeyan, 2010; Bondock *et al*.,

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2013; Karuvalam *et al*., 2012). After the discovery of pyrazofurin (a potent antimicrobial), usefulness of pyrazole derivatives in the field of medicine has been much explored (Manojkumar *et al*., 2009a). An extensive research in this field revealed that 4-arylazo-substituted pyrazole derivatives were found to act as analgesic (Oru *et al*., 2006), cytotoxic (Manojkumar *et al*., 2009b), antistaphylococcal (Raimondi *et al*., 2012), antioxidant (Manojkumar *et al*., 2009a) and CDK2-cyclin E-inhibiting agents (Krystof *et al*., 2006). In the literature, 3,5-dimethyl-substituted 4-arylazopyrazole derivatives were also reported to exhibit high antimicrobial and antibacterial potential (Manojkumar *et al*., 2009a; Kale, 2013; Javed and Hassan, 2013) (Fig. 1). Moreover, pyrazole-linked thiazoles exhibited antibacterial (Aggarwal *et al*., 2011), antimicrobial (Mor *et al*., 2012; Prakash *et al*., 2014) and ΔF508-CFTR corrector activities (Ye *et al*., 2010) besides treating cardiovascular diseases (Sanfilippo *et al*., 1995). Some important examples of pyrazolylthiazole derivatives associated with significant antibacterial (Aggarwal *et al*., 2011) and antimicrobial profile (Mor *et al*., 2012; Prakash *et al*., 2014) are shown in Fig. 2.

On the other hand, a great attention has been paid worldwide toward the developments of DNA damage-protecting and DNA-photocleaving agents (Deryabin *et al*., 2012; Toshima *et al*., 2002). It has been reported that exposure to UV radiations resulted in serious disorders like erythema, edema, hyperpigmentation, immunosuppression, photoaging, skin cancer, cataract formation and retinal degeneration (Perez-Sanchez *et al*., 2014; Holloy, 2002). Irradiation of UV radiation is quite responsible for the generation of various radicals like reactive oxygen species (ROS) and reactive nitrogen species (RNS) which may further affect a number of biomolecules (Perez-Sanchez *et al*., 2014; Holloy, 2002). These radiations lead to the formation of intra- and intermolecular cross-links in DNA besides its degradation (Cai *et al*., 1998). One of the most common problems encountered among the genetic engineers was inability to find the exact results while performing the gel electrophoresis experiments (Deryabin *et al*., 2012). A long-time exposure of gel to UV light resulted in the degradation of DNA bands. Therefore, protection of DNA from UV radiations is not only important for the viability of living systems but also important for the effectiveness of DNA-based laboratory techniques (Deryabin *et al*., 2012). Though in recent years some heterocyclic or non-heterocyclic compounds have been used to protect the DNA from UV-induced damage, developments of newer and efficient protecting agents are still needed (Deryabin *et al*., 2012). Furthermore, photocleaving agents generate some structural modifications in DNA under UV irradiation and thus contributed in

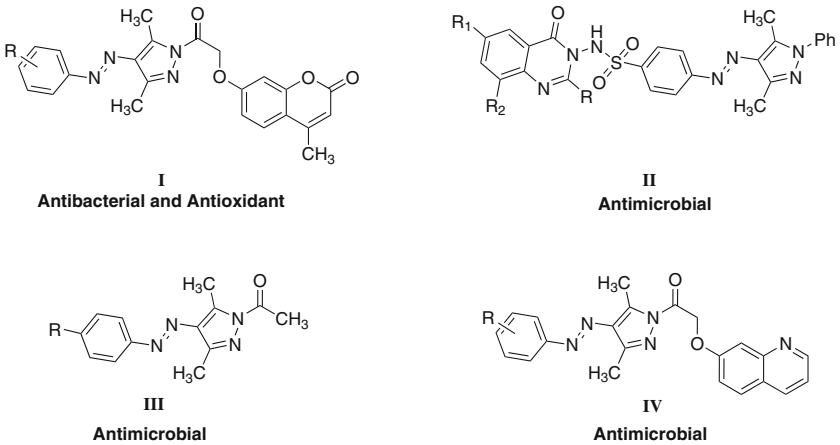


Fig. 1 4-Arylazo-3,5-dimethylpyrazoles **(I–IV)** associated with antimicrobial, antibacterial and antioxidant property

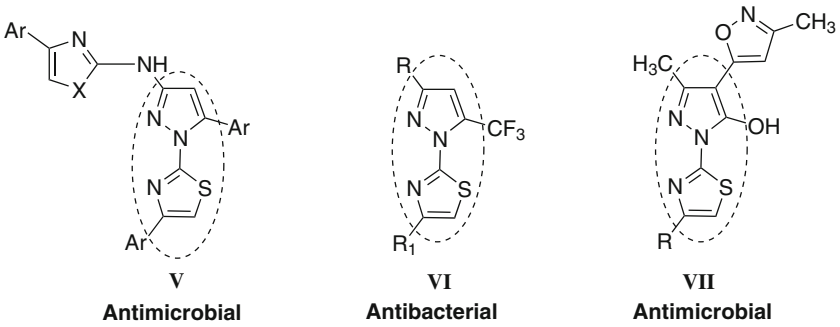


Fig. 2 Some pyrazolylthiazoles **(V–VII)** as antimicrobial/antibacterial agents

development of antitumor drugs (Toshima *et al*., 2002). The literature survey revealed that compounds bearing pyrazole (Kumar *et al*., 2014b; Kamal *et al*., 2014) and thiazole (Li *et al*., 2004, 2005) nuclei act as efficient DNA-photocleaving agents.

In addition, free radicals generated either by UV light or via metabolic processes in living systems lead to various serious disorders like coronary heart diseases, ulcers, cancers and neurodegenerative diseases besides damaging DNA, proteins and lipids (Kalita *et al*., 2012; Emen *et al*., 2009). Though nature already provided each cell a protective mechanism (known as antioxidant mechanism), administration of antioxidants played an important role in protecting the biological targets when the normal antioxidant defense mechanism fails (Kalita *et al*., 2012). The antioxidants inhibit free radicals and stop the free radical chain reaction by donating an electron or hydrogen atom (Gulcin, 2012). It has already been reported that some 3,5-dimethyl-4-arylazopyrazole derivatives (Manojkumar *et al*., 2009a) **(I)** (Fig. 1) and thiazole derivatives were proved as an efficient class of antioxidants (Andreani *et al*., 2013).

Prompted from the above facts and in continuation to our work related to the greener synthetic approaches toward biologically active compounds (Kumar *et al*., 2014b; Gupta *et al*., 2014), it was planned to synthesize some novel 4-aryl-2-(3,5-dimethyl-1*H*-pyrazol-1-yl)thiazole derivatives bearing arylazo group at position-4 of the pyrazole moiety under solvent-free conditions. The two main objectives of the study are: a) to observe the influence of 4-arylazo group on the Hantzsch thiazole approach in both the solvent-free as well as solvent-mediated conditions, and b) to explore the antimicrobial, antioxidant and UV-mediated DNA damage-protective as well as DNA photocleavage potential with an expectation to find a new class of bioactive compounds.

Materials and methods

Chemistry

Melting points were determined by open capillary method and are uncorrected. The FT-IR spectra of the compounds were recorded on the FT-Infrared Spectrometer Model RZX (PerkinElmer) using KBr pellets. The ^{1}H and ^{13}C NMR spectra were recorded on the Bruker Advance II 400 NMR Spectrometer at 400 MHz and 100 MHz, respectively; chemical shifts are expressed on δ-scale downfield from TMS as an internal standard. Thermo Scientific (FLASH 2000) CHN Elemental Analyzer was used to determine percentages of C, H and N with an accuracy of 0.3 %. The absorbance for the antioxidant activity was recorded on specord 250 analytikjena UV spectrophotometer. The reactants (*E*)-3,5-dimethyl-4-(aryldiazenyl)-1*H*-pyrazole-1-carbothioamides **1** (Kumar *et al*., 2006; Garg and Sharma, 1969) and α-bromoketones **2** (Langley, 1929, 1941) were synthesized according to the literature procedure.

Synthesis of (*E*)-2″-(3,5-dimethyl-4-(aryldiazenyl)-1*H*-pyrazol-1-yl)-4″-arylthiazoles 3a–p

General procedure

In a dried mortar, a mixture of (*E*)-3,5-dimethyl-4-(*p*-tolyldiazenyl)-1*H*-pyrazole-1-carbothioamide **1** (0.1 mol), phenacyl bromide **2a** (0.1 mol) and sodium carbonate (0.6 mol) was ground for 10–15 min at 100 °C. After that, the reaction mixture was poured into water to remove the sodium carbonate. The obtained crude product was recrystallized from ethanol.

(E)-2″-(3,5-Dimethyl-4-(p-tolyldiazenyl)-1H-pyrazol-1-yl)-4″-phenylthiazole ***3a*** Yield: 82 %; m.p. 206–207 °C; IR (ν_{max}, cm^{-1}): 1697 (C=N str.), 1566 (C=C str.); ^{1}H NMR (400 MHz; $CDCl_3$, δ_H): 2.36 (s, 3H, 4′-C**H$_3$**), 2.51 (s, 3H, 3-C**H$_3$**), 3.09 (s, 3H, 5-C**H$_3$**), 7.21 (s, 1H, 5″-**H**), 7.22 (d, 2H, 3′, 5′-**H**, $^{3}J_{H\text{-}H}$ = 8.12 Hz), 7.29–7.31 (m, 1H, 4‴-**H**), 7.36–7.40 (m, 2H, 3‴, 5‴-**H**), 7.68 (d, 2H, 2′, 6′-**H**, $^{3}J_{H\text{-}H}$ = 8.24 Hz), 7.84–7.86 (m, 2H, 2‴, 6‴-**H**); Anal. calcd for $C_{21}H_{19}N_5S$ (%): C, 67.56; H, 5.09; N, 18.77. Found (%): C, 67.52; H, 5.08; N, 18.72.

(E)-4″-(4‴-Bromophenyl)-2″-(3,5-dimethyl-4-(p-tolyldiazenyl)-1H-pyrazol-1-yl)thiazole ***3b*** Yield: 83 %; m.p. 214–216 °C; IR (ν_{max}, cm^{-1}): 1692 (C=N str.), 1563 (C=C str.); ^{1}H NMR (400 MHz; $CDCl_3$ + TFA, δ_H): 2.53 (s, 3H, 4′-C**H$_3$**), 2.87 (s, 3H, 3-C**H$_3$**), 3.29 (s, 3H, 5-C**H$_3$**), 7.47 (d, 2H, 3′, 5′-**H**, $^{3}J_{H\text{-}H}$ = 8.12 Hz), 7.64 (d, 2H, 3‴, 5‴-**H**, $^{3}J_{H\text{-}H}$ = 8.12 Hz), 7.65 (s, 1H, 5″-**H**), 7.78–7.84 (m, 4H, 2′, 6′, 2″, 6″-**H**); Anal. calcd for $C_{21}H_{18}BrN_5S$ (%): C, 55.63; H, 3.97; N, 15.45. Found (%): C, 55.61; H, 3.95; N, 15.41.

(E)-4″-(4‴-Chlorophenyl)-2″-(3,5-dimethyl-4-(p-tolyldiazenyl)-1H-pyrazol-1-yl)thiazole ***3c*** Yield: 84 %; m.p. 210–211 °C; IR (ν_{max}, cm^{-1}): 1693 (C=N str.), 1563 (C=C str.); ^{1}H NMR (400 MHz; $CDCl_3$ + DMSO-d_6, δ_H): 2.53 (s, 3H, 4′-C**H$_3$**), 2.56 (s, 3H, 3-C**H$_3$**), 3.14 (s, 3H, 5-C**H$_3$**), 7.30–7.44 (m, 2H, 3′, 5′-**H**), 7.66 (s, 1H, 5″-**H**), 7.72–7.74 (m, 2H, 3‴, 5‴-**H**), 7.95 (m, 4H, 2′, 6′, 2″, 6″-**H**); Anal. calcd for $C_{21}H_{18}ClN_5S$ (%): C, 61.84; H, 4.42; N, 17.18. Found (%): C, 61.82; H, 4.41; N, 17.13.

(E)-2″-(3,5-Dimethyl-4-(p-tolyldiazenyl)-1H-pyrazol-1-yl)-4″-(4‴-fluorophenyl)thiazole ***3d*** Yield: 82 %; m.p. 184–185 °C; IR (ν_{max}, cm^{-1}): 1695 (C=N str.), 1565 (C=C str.); ^{1}H NMR (400 MHz; $CDCl_3$, δ_H): 2.31 (s, 3H, 4′-C**H$_3$**),

2.46 (s, 3H, 3-C**H$_3$**), 3.01 (s, 3H, 5-C**H$_3$**), 7.00–7.04 (m, 3H, 3‴, 5‴-**H**), 7.05 (s, 1H, 5″-**H**), 7.17 (d, 2H, 3′, 5′-H, $^3J_{H\text{-}H}$ = 8.24 Hz), 7.63 (d, 2H, 2′, 6′-**H**, $^3J_{H\text{-}H}$ = 7.96 Hz), 7.74–7.77 (m, 2H, 2‴, 6‴-**H**); Anal. calcd for $C_{21}H_{18}FN_5S$ (%): C, 64.45; H, 4.60; N, 17.90. Found (%): C, 64.44; H, 4.56; N, 17.88.

(E)-2″-(3,5-Dimethyl-4-(p-tolyldiazenyl)-1H-pyrazol-1-yl)-4″-p-tolylthiazole ***3e*** Yield: 84 %; m.p. 187–189 °C; IR (ν_{max}, cm^{-1}): 1698 (C=N str.), 1565 (C=C str.); ^{1}H NMR (400 MHz; $CDCl_3$, δ_H): 2.40 (s, 3H, 4‴-C**H$_3$**), 2.43 (s, 3H, 4′-C**H$_3$**), 2.58 (s, 3H, 3-C**H$_3$**), 3.16 (s, 3H, 5-C**H$_3$**), 7.21 (s, 1H, 5″-**H**), 7.25 (d, 2H, 3‴, 5‴-**H**, $^3J_{H\text{-}H}$ = 8.12 Hz), 7.29 (d, 2H, 3′, 5′-**H**, $^3J_{H\text{-}H}$ = 8.20 Hz), 7.75 (d, 2H, 2′, 6′-**H**, $^3J_{H\text{-}H}$ = 8.20 Hz), 7.81(d, 2H, 2‴, 6‴-**H**, $^3J_{H\text{-}H}$ = 8.12 Hz); Anal. calcd for $C_{22}H_{21}N_5S$ (%): C, 68.22; H, 5.43; N, 18.09. Found (%): C, 68.20; H, 5.41; N, 18.07.

2″-(3,5-Dimethyl-4-(p-tolyldiazenyl)-1H-pyrazol-1-yl)-4″-(naphthalen-2‴-yl)thiazole ***3f*** Yield: 78 %; m.p. 176–179 °C; IR (ν_{max}, cm^{-1}): 1696 (C=N str.), 1566 (C=C str.); ^{1}H NMR (400 MHz; $CDCl_3$, δ_H): 2.40 (s, 3H, 4′-C**H$_3$**), 2.55 (s, 3H, 3-C**H$_3$**), 3.15 (s, 3H, 5-C**H$_3$**), 7.26 (d, 2H, 3′, 5′-**H**, $^3J_{H\text{-}H}$ = 7.80 Hz), 7.31 (s, 1H, 5″-**H**), 7.45–7.97 (m, 6H, 3‴, 4‴, 5‴, 6‴, 7‴, 8‴-**H**), 7.73 (d, 2H, 2′, 6′-**H**, $^3J_{H\text{-}H}$ = 7.80 Hz), 8.34 (s, 1H, 1‴-**H**); Anal. calcd for $C_{25}H_{21}N_5S$ (%): C, 70.92; H, 4.96; N, 16.55. Found (%): C, 70.90; H, 4.92; N, 16.52.

(E)-2″-(3,5-Dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)-4″-phenylthiazole ***3g*** Yield: 80 %; m.p. 168–170 °C; IR (ν_{max}, cm^{-1}): 1694 (C=N str.), 1564 (C=C str.); ^{1}H NMR (400 MHz; $CDCl_3$, δ_H): 2.57 (s, 3H, 3-C**H$_3$**), 3.15 (s, 3H, 5-C**H$_3$**), 7.23 (s, 1H, 5″-**H**), 7.32–7.36 (m, 1H, 4‴-**H**), 7.38–7.49 (m, 5H, 3′, 5′, 4′, 3‴, 5‴-**H**), 7.83 (d, 2H, 2′, 6′-**H**, $^3J_{H\text{-}H}$ = 7.52 Hz), 7.89 (d, 2H, 2‴, 6‴-**H**, $^3J_{H\text{-}H}$ = 7.40 Hz); Anal. calcd for $C_{20}H_{17}N_5S$ (%): C, 66.85; H, 4.74; N, 19.50. Found (%): C, 66.80; H, 4.70; N, 19.48.

(E)-4″-(4‴-Bromophenyl)-2″-(3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)thiazole ***3h*** Yield: 84 %; m.p. 200–201 °C; IR (ν_{max}, cm^{-1}): 1698 (C=N str.), 1569 (C=C str.); ^{1}H NMR (400 MHz; $CDCl_3$, δ_H): 2.58 (s, 3H, 3-C**H$_3$**), 3.15 (s, 3H, 5-C**H$_3$**), 7.27 (s, 1H, 5″-**H**), 7.40–7.44 (m, 1H, 4′-**H**), 7.48–7.51 (m, 2H, 3′, 5′-**H**), 7.55–7.58 (m, 2H, 3‴, 5‴-**H**), 7.76–7.79 (m, 2H, 2′, 6′-**H**), 7.83–7.85 (m, 2‴, 6‴-**H**); Anal. calcd for $C_{20}H_{16}BrN_5S$ (%): C, 54.67; H, 3.64; N, 15.95. Found (%): C, 54.65; H, 3.61; N, 15.92.

(E)-4″-(4‴-Chlorophenyl)-2″-(3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)thiazole ***3i*** Yield: 85 %; m.p. 188–189 °C; IR (ν_{max}, cm^{-1}): 1698 (C=N str.), 1566 (C=C str.); ^{1}H NMR (400 MHz; $CDCl_3$, δ_H): 2.56 (s, 3H, 3-C**H$_3$**), 3.12 (s, 3H, 5-C**H$_3$**), 7.21 (s, 1H, 5″-**H**), 7.37–7.42 (m, 3H, 4′, 3‴, 5‴-**H**), 7.46–7.50 (m, 2H, 3′, 5′-**H**), 7.79–7.84 (m, 4H, 2′, 6′, 2‴, 6‴-**H**); Anal. calcd for $C_{20}H_{16}ClN_5S$ (%): C, 61.07; H, 4.07; N, 17.79. Found (%): C, 61.04; H, 4.08; N, 17.75.

(E)-2″-(3,5-Dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)-4″-(4‴-fluorophenyl)thiazole ***3j*** Yield: 83 %; m.p. 164–165 °C; IR (ν_{max}, cm^{-1}): 1697 (C=N str.), 1566 (C=C str.); ^{1}H NMR (400 MHz; $CDCl_3$, δ_H): 2.54 (s, 3H, 3-C**H$_3$**), 3.11 (s, 3H, 5-C**H$_3$**), 7.07–7.12 (m, 2H, 3‴, 5‴-**H**), 7.13 (s, 1H, 5″-**H**), 7.37–7.41 (m, 1H, 4′-**H**), 7.45–7.48 (m, 2H, 3′, 5′-**H**), 7.80–7.85 (m, 2′, 6′, 4″, 2‴, 6‴-**H**); Anal. calcd for $C_{20}H_{16}FN_5S$ (%): C, 63.66; H, 4.24; N, 18.57. Found (%): C, 63.61; H, 4.22; N, 18.55.

(E)-2″-(3,5-Dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)-4″-p-tolylthiazole ***3k*** Yield: 87 %; m.p. 167 °C; IR (ν_{max}, cm^{-1}): 1698 (C=N str.), 1568 (C=C str.); ^{1}H NMR (400 MHz; $CDCl_3$, δ_H): 2.27 (s, 3H, 4‴-C**H$_3$**), 2.46 (s, 3H, 3-C**H$_3$**), 3.03 (s, 3H, 5-C**H$_3$**), 7.05 (s, 1H, 5″-**H**), 7.12 (d, 2H, 3‴, 5‴-**H**, $^3J_{H\text{-}H}$ = 8.00 Hz), 7.28–7.31 (m, 1H, 4′-**H**), 7.35–7.39 (m, 2H, 3′, 5′-**H**), 7.67 (d, 2H, 2‴, 6‴-**H**, $^3J_{H\text{-}H}$ = 8.00 Hz), 7.72 (d, 2H, 2′, 6′-**H**, $^3J_{H\text{-}H}$ = 7.56 Hz); Anal. calcd for $C_{21}H_{19}N_5S$ (%): C, 67.56; H, 5.09; N, 18.77. Found (%): C, 67.55; H, 5.03; N, 18.72.

2″-(3,5-Dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)-4″-(naphthalen-2‴-yl)thiazole ***3l*** Yield: 80 %; m.p. 180–181 °C; IR (ν_{max}, cm^{-1}): 1698 (C=N str.), 1567 (C=C str.); ^{1}H NMR (400 MHz; $CDCl_3$, δ_H): 2.59 (s, 3H, 3-C**H$_3$**), 3.21 (s, 3H, 5-C**H$_3$**), 7.37 (s, 1H, 5″-**H**), 7.40–7.44 (m, 1H, 4′-**H**), 7.48–7.98 (m, 10H, 2′, 3′, 5′, 6′, 3‴, 4‴, 5‴, 6‴, 7‴, 8‴-**H**), 8.39 (s, 1H, 1‴-**H**); Anal. calcd for $C_{24}H_{19}N_5S$ (%): C, 70.42; H, 4.65; N, 17.11. Found (%): C, 70.39; H, 4.62; N, 17.07.

(E)-2″-(4-((4′-Fluorophenyl)diazenyl)-3,5-dimethyl-1H-pyrazol-1-yl)-4″-phenylthiazole ***3m*** Yield: 82 %; m.p. 194 °C; IR (ν_{max}, cm^{-1}): 1696 (C=N str.), 1562 (C=C str.); ^{1}H NMR (400 MHz; $CDCl_3$, δ_H): 2.56 (s, 3H, 3-C**H$_3$**), 3.15 (s, 3H, 5-C**H$_3$**), 7.14–7.19 (m, 2H, 3′, 5′-**H**), 7.27 (s, 1H, 5″-**H**), 7.34–7.37 (m, 1H, 4‴-**H**), 7.43–7.47 (m, 2H, 3‴, 5‴-**H**), 7.83–7.86 (m, 2H, 2′, 6′-**H**), 7.90–7.92 (m, 2H, 2‴, 6‴-**H**); Anal. calcd for $C_{20}H_{16}$ FN_5S (%): C, 63.66; H, 4.24; N, 18.57. Found (%): C, 63.61; H, 4.22; N, 18.52.

(E)-4″-(4‴-Bromophenyl)-2″-(4-((4′-fluorophenyl)diazenyl)-3,5-dimethyl-1H-pyrazol-1-yl)thiazole ***3n*** Yield: 86 %; m.p. 201–202 °C; IR (ν_{max}, cm^{-1}): 1698 (C=N str.), 1562 (C=C str.); ^{1}H NMR (400 MHz; $CDCl_3$, δ_H): 2.56 (s, 3H, 3-C**H$_3$**), 3.13 (s, 3H, 5-C**H$_3$**), 7.14–7.19 (m, 2H, 3′, 5′-**H**), 7.26 (s, 1H, 5″-**H**), 7.54–7.58 (m, 2H, 3‴, 5‴-**H**), 7.75–7.78 (m, 2H, 2‴, 6‴-**H**), 7.83–7.86 (m, 2H, 2′, 6′-**H**); Anal. calcd for $C_{20}H_{15}BrFN_5S$ (%): C, 52.52; H, 3.28; N, 15.32. Found (%): C, 52.48; H, 3.26; N, 15.29.

(E)-4″-(4‴-Chlorophenyl)-2″-(4-((4′-fluorophenyl)diazenyl)-3,5-dimethyl-1H-pyrazol-1-yl)thiazole ***3o*** Yield: 86 %; m.p. 198 °C; IR (ν_{max}, cm^{-1}): 1698 (C=N str.), 1562 (C=C str.); ^{1}H NMR (400 MHz; $CDCl_3$, δ_H): 2.55 (s, 3H, 3-C**H**$_3$), 3.12 (s, 3H, 5-C**H**$_3$), 7.14–7.18 (m, 2H, 3′, 5′-**H**), 7.24 (s, 1H, 5″-**H**), 7.38–7.42 (m, 2H, 3‴, 5‴-**H**), 7.80–7.85 (m, 4H, 2′, 6′, 2‴, 6‴-**H**); Anal. calcd for $C_{20}H_{15}ClFN_5S$ (%): C, 58.32; H, 3.65; N, 17.01. Found (%): C, 58.29; H, 3.62; N, 17.00.

(E)-4″-(4‴-Fluorophenyl)-2″-(4-((4′-fluorophenyl)diazenyl)-3,5-dimethyl-1H-pyrazol-1-yl)thiazole ***3p*** Yield: 81 %; m.p. 197–198 °C; IR (ν_{max}, cm^{-1}): 1694 (C=N str.), 1562 (C=C str.); ^{1}H NMR (400 MHz; $CDCl_3$, δ_H): 2.54 (s, 3H, 3-C**H**$_3$), 3.11 (s, 3H, 5-C**H**$_3$), 7.09–7.17 (m, 4H, 3′, 5′, 3‴, 5‴-**H**), 7.16 (s, 1H, 5″-**H**), 7.80–7.87 (m, 4H, 2‴, 6‴, 2′, 6′-**H**); Anal. calcd for $C_{20}H_{15}F_2N_5S$ (%): C, 60.76; H, 3.80; N, 17.72. Found (%): C, 60.73; H, 3.76; N, 17.70.

Biological activity

Antimicrobial activity

Test microorganisms: On the basis of clinical importance in humans, total six microbial strains were selected. These strains include two Gram-positive bacteria, viz. *Staphylococcus aureus* MTCC 96 and *Bacillus subtilis* MTCC 121, two Gram-negative bacteria, viz. *Escherichia coli* MTCC 1652 and *Pseudomonas aeruginosa* MTCC 741, and two yeasts namely *Candida albicans* MTCC 227 and *Saccharomyces cerevisiae* MTCC 170. All the microbial cultures were purchased from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh. Subculturing of bacterial strains was done on nutrient agar (NA), while yeasts were carried on malt extract agar (MEA) plates.

Determination of zone of inhibition

The zone of inhibition of all the synthesized compounds was measured by the agar well diffusion assay (Kamal *et al*., 2015). The inoculum suspensions of the test microorganisms were prepared by using 16 h old cultures adjusted to 10^8 cfu/mL by referring the 0.5 McFarland standards. Total 20 mL of agar medium (NA in case of antibacterial and MEA in case of antifungal) was poured into each petri plate, and then plates were swabbed with 100 µL inocula of the test microorganisms and kept for 15 min for adsorption. Wells were bored into the seeded agar plates using a sterile cork borer of diameter (8 mm), and these were loaded with a 100 µL volume with concentration of 4.0 mg/mL of each compound reconstituted in the dimethylsulfoxide (DMSO). The incubation of all the plates was carried at 37 °C for 24 h. Antimicrobial activity of each compound against the selected organisms was evaluated by measuring the zone of inhibition with zone reader (Hi antibiotic zone scale). Ciprofloxacin and amphotericin-B were used as positive control for bacterial and yeast strains, respectively. This procedure was performed in three replicate plates for each organism.

Determination of minimum inhibitory concentration (MIC)

MIC is the lowest concentration of an antimicrobial compound that inhibits the visible growth of the microorganisms after incubation (Gulcin *et al*., 2008, 2010). MIC of the various compounds against bacterial and yeast strains was tested through a modified agar well diffusion method (Kamal *et al*., 2015). In this protocol, a twofold serial dilution of each compound was prepared by first reconstituting the compound in DMSO followed by dilution in sterile distilled water to achieve a decreasing concentration range of 4–0.0625 mg/mL. A 100 µL volume of each dilution was introduced into wells (in triplicate) in the agar plates already seeded with 100 µL of standardized inoculum (10^8 cfu/mL) of the test microbial strain. All test plates were incubated aerobically at 37 °C for 24 h and observed for the inhibition zones. MIC was taken as the lowest concentration of the chemical compound that inhibited the growth of the microbes which was shown by a clear zone of inhibition and was recorded for each test organism. Ciprofloxacin and amphotericin-B were used as positive controls, while DMSO was used as a negative control in this investigation.

Free radical scavenging of the compounds using DPPH analysis

The DPPH free radical-scavenging activity is based on the fact that methanolic solution of DPPH, which imparts vivid purple color and gives strong absorption band at 515 nm, gets reduced in the presence of an antioxidant compound (Bondet *et al*., 1997). Different concentrations of the compounds under evaluation (50–400 µg/mL) were added to 4 mL of a DPPH solution (120 µM) in methanol and incubated at 37 °C temperature for 30 min in dark. The absorbance was determined at 515 nm, and the percentage free radical scavenging (%) was calculated according to the following equation:

$$\text{Scavenging}\,\% = \left[\left(A_{\text{control}} - A_{\text{sample}}\right)/A_{\text{control}}\right] \times 100$$

where A_{control} is the absorbance of the control reaction (containing all reagents except the test compounds) and A_{sample} is the absorbance of the test compound. Ascorbic acid was used as a positive control, and tests were conducted in triplicate.

UV-mediated plasmid DNA damage-protecting/ DNA photocleavage activity

Treatment of plasmid DNA with the samples

The stock solutions for all tested compounds were prepared by dissolving 0.005 g of compound in 0.5 mL of DMSO. All synthesized compounds **3a**–**p** (40 μg) in DMSO were added separately to volume of 2 μL containing plasmid DNA in TE (*Tris* 10 mM, EDTA 0.01 mM, pH 8.0) buffer. The same volume of DMSO as used to make the solution of the test compounds was added into **A** and control **C**. The reaction volumes except **A** were held in caps of polyethylene microcentrifuge tubes, which were irradiated directly on the surface of a transilluminator (8000 mW/cm) at 360 nm for 1 h at room temperature. After that, **A**, control (**C**) and test samples were incubated at 37 °C for 0.5 h.

Agarose gel electrophoresis

After the treatment, electrophoresis was done according to the given procedure as mentioned below (Taj *et al*., 2011):

To a 2 mL 50X Tris-acetate-EDTA buffer (TAE) (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH:8. 0), 98 mL of autoclaved water was added to make it 1X TAE buffer solution. Agarose (0.8 g) was dissolved on boiling the resultant mixture. When the gel attained 55 °C temperature, 10 mg/mL of ethidium bromide (EtBr) was added. The treated DNA mixed with 6X loading dye (0.25 %) bromophenol blue was added, and then it was poured into gel cassette fitted with a comb. The gel was then allowed to solidify. The comb was carefully removed, and the gel was placed over electrophoresis chamber flooded with Tris-acetate-EDTA buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH:8.0 and 30 % glycerol) and was carefully loaded into the wells along with the control (**C**) and **A**, and electrophoresis was carried out at 5 V/cm for 2.0 h, and the bands were observed under UV transilluminator.

Results and discussion

Chemistry

The synthesis of novel (*E*)-2-(3,5-dimethyl-4-(aryldiazenyl)-1*H*-pyrazol-1-yl)-4-arylthiazoles **3a**–**p** has been accomplished via Hantzsch thiazole approach under solvent-free conditions (Scheme 1).

To achieve the product, (*E*)-2″-(3,5-dimethyl-4-(*p*-tolyldiazenyl)-1*H*-pyrazol-1-yl)-4″-phenylthiazole **3a**, the reactant (*E*)-3,5-dimethyl-4-(*p*-tolyldiazenyl)-1*H*-pyrazole-1-carbothioamide **1a** was ground with phenacyl bromide **2a** in the presence of sodium carbonate at 100 °C temperature. The product **3a** of high purity was formed within 10–15 min under solvent-free conditions (Scheme 1). The starting precursors, **1** (Kumar *et al*., 2006; Garg and Sharma, 1969) and **2**, were synthesized by the reported methods (Langley, 1941; Langley, 1929). Various primary aromatic amines on diazotization followed by coupling with acetyl acetone yielded 3-arylazopentane-2,4-diones which on treatment with thiosemicarbazide afforded **1**. The structure of **3a** was established on the basis of a combined use of IR, NMR (^{1}H and ^{13}C), COSY, ROESY, HSQC and HMBC spectroscopy.

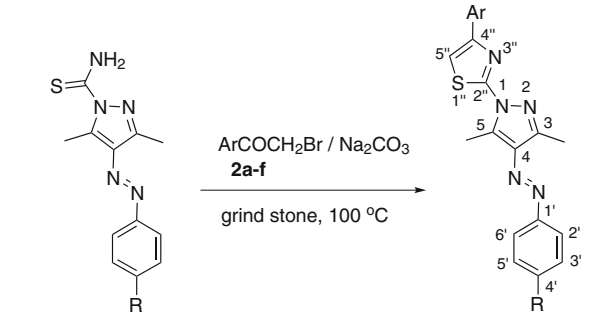
The disappearance of N–H stretching bands at 3140 and 3387 cm^{-1} due to NH_2 group of carbothioamide indicated the formation of compound **3a**. In ^{1}H NMR spectrum, disappearance of two signals at δ 6.96 and 8.69 for NH_2 protons and an appearance of singlet at δ 7.21 due to thiazole proton (H-5″) confirmed the formation of **3a**. The structure of **3a** was further supported by its ^{13}C NMR spectrum in which three characteristic signals of thiazole system at δ 161.67, 152.79 and 109.10 were appeared due to C-2′, C-4′ and C-5′, respectively.

The chemical shifts for four methyl protons of **3e** were assigned by analyzing its ^{1}H-^{1}H COSY, HMQC and HMBC spectral data. In the COSY spectrum, 3‴, 5‴-H protons have shown a correlation with 2‴, 6‴-H protons and vice - versa, while 3′, 5′-H protons showed the correlation with 2′, 6′-H protons. The HMBC spectrum revealed that the protons 3‴, 5‴-H have shown a correlation with 4‴-CH_3 protons, while protons 3′, 5′-H showed a correlation with 4′-CH_3 protons. The 4‴-CH_3 protons exhibited the correlation with C-3‴, C-5‴, C-4‴ and C-1‴ carbons, while the correlation of 4″-CH_3 protons was observed with C-3″, C-5″, C-4″ and C-1″ carbons. A correlation of 3′-CH_3 protons with C-3′ and C-4′ carbons has also been observed. The 5′-CH_3 protons were correlated with C-4′ and C-5′ carbons, and a correlation of 5′-carbon with CH_2 protons was also observed. In the ROSEY spectrum, the protons 3′ and 5′-H have shown a strong spacial relationship with 4′-CH_3 protons, while protons 3‴ and 5‴-H showed the spacial relationship with 4‴-CH_3 protons.

In order to generalize the protocol, various α-bromoketones **2** were treated with different pyrazole-1-carbothioamides **1** under the similar reaction conditions to achieve pyrazol-1-ylthiazoles **3**. The ^{13}C NMR spectral results of all the synthesized compounds **3a**–**p** are presented in Tables 1 and 2.

Initially, in the present investigation, an attempt to perform the reaction of **1** with **2a** in ethanol under reflux conditions has been made which resulted in an exclusive formation of a mixture of thiocyanatoketone **4** and cleaved pyrazole **5**. The results were supported by the appearance

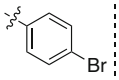
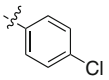
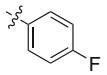
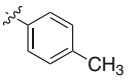
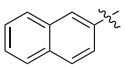
Scheme 1 Solvent-free synthesis of (*E*)-2-(3,5-dimethyl-4-(aryldiazenyl)-1*H*-pyrazol-1-yl)-4-arylthiazoles **3a–p**



1a-c **3a-p**

For compds (**1**) **a b c**
R = CH_3, H, F

For compds **(3a-f), R = CH_3**
(3g-l), R = H
(3m-p), R = F

For compds (2, 3)	a g m	b h n	c i o	d j p	e k	f l
Ar						

of a characteristic signal at δ 4.72 (s, 2H, CH_2) of **4** as well as a signal at δ 2.52 (s, 6H, 3,5-CH_3) and 2.34 (s, 3H, 4′-CH_3) of **5** in the ^{1}H NMR spectrum of the crude reaction mixture (Scheme 2). This observation was consistent with the previously reported results based on the reaction of 4-unsubstituted pyrazole-1-carbothioamide (Gupta *et al.*, 2014). In this study, it has been found that arylazo group present at position-4 of the pyrazole moiety did not play any role in preventing the cleavage of C-N bond in the reaction of differently substituted pyrazole-1-carbothioamides with α-bromoketones.

Biological activity

Antimicrobial evaluation

To explore the antimicrobial potential, sixteen newly synthesized compounds were screened for their in vitro antibacterial and antifungal activity through agar-diffusion method using ciprofloxacin and amphotericin-B as positive controls for bacteria and yeasts, respectively. The preliminary results were recorded by measuring the inhibition zones (IZ) of bacterial or fungal growth around the wells for each tested compound at 400 µg/100 µL. From the preliminary study, it has been found that all the compounds (except **3c** and **3e**) were found active against the yeast strain, viz. *C. albicans* (IZ in the diameter range = 12–50 mm) (Table 3; Fig. 3). Among them, compound **3l** showed a very big inhibitory zone of diameter 50 mm, while **3g–k** displayed zones of 21–25 mm in reference to amphotericin-B, the standard drug (IZ = 16.6 mm). Three compounds **3a**, **3b** and **3d** were found active against *E. coli* (IZ = 12 mm), and none of the compounds possess activity against *B. subtilis*, *S. aureus*, *P. aeruginosa* and *S. cerevisiae*. The minimum inhibitory concentration (MIC) in µg/100 µL was measured using twofold serial dilution method for those compounds which have displayed appreciable inhibitory zones (>16 mm) (Table 4).

From the MIC results, it has been found that **3l** exhibited two times high inhibitory potential (MIC = 6.25) against *C. albicans* in comparison with the standard drug (MIC = 12.5), while compound **3k** (MIC = 12.5) was found equipotent. On the other hand, the compounds **3g–j** exhibited two-fold lesser inhibitory action with MIC = 25 against *C. albicans* as compared to the reference drug.

The results drawn from the antimicrobial screening (on the basis of MIC value) demonstrated the following assumptions about the structure–activity relationship (SAR).

- Most of the synthesized compounds were found active selectively against the fungal strain, *C. albicans*.
- It has been observed that unsubstituted phenyl ring (R = H) of the arylazo moiety may be responsible for the higher antifungal potential.
- Substitution on position-4 of the phenyl ring in arylazo group (R = CH_3 and F) leads to diminish the antifungal potential.
- Among active compounds, antifungal potential was found to be higher than that of the standard drug,

Table 1 ^{13}C NMR data of the synthesized compounds **3a–h**

Compounds[a]	3a	3b	3c	3d	3e	3f	3g	3h
C-3	145.28	146.60	145.40	145.33	145.20	145.32	145.25	145.43
CH_3-3	14.78	13.60	14.76	14.72	14.70	14.75	14.76	14.73
C-4	137.17	134.16	137.19	137.15	137.10	137.16	137.23	137.29
C-5	141.46	146.00	141.38	141.26	141.30	141.34	141.79	141.73
CH_3-5	12.06	12.14	12.04	12.01	12.00	12.09	12.06	12.03
C-1′	151.49	150.80	151.44	151.46	151.50	151.50	153.40	153.37
C-2′	122.06	120.89	122.07	122.05	122.00	122.07	122.12	122.11
C-3′	129.67	131.17	129.67	129.63	129.60	129.64	129.00	129.02
C-4′	140.52	140.07	140.57	140.51	140.40	140.47	130.09	130.16
C-5′	129.67	131.17	129.67	129.63	129.60	129.64	129.00	129.02
C-6′	122.06	120.89	122.07	122.05	122.00	122.07	122.12	122.11
CH_3-4′	21.45	21.07	21.46	21.44	21.40	21.46	–	–
C-2″	161.67	157.65	161.88	161.81	161.50	161.72	161.60	161.87
C-4″	152.79	153.05	152.61	151.74	152.90	152.74	152.81	151.70
C-5″	109.10	113.37	109.40	108.55	108.30	109.46	109.15	109.61
C-1‴	134.17	132.19	132.60	130.46 (d, $^4J_{C\text{-}F} = 3.02$ Hz)	131.51	125.07[b]	134.16	133.08
C-2‴	126.03	127.61	127.25	127.73 (d, $^3J_{C\text{-}F} = 9.05$ Hz)	125.90	131.50[b]	126.03	127.57
C-3‴	128.81	131.25	128.96	115.68 (d, $^2J_{C\text{-}F} = 22.13$ Hz)	129.40	123.88[b]	128.79	131.92
C-4‴	128.31	123.56	134.08	162.75 (d, $^1J_{C\text{-}F} = 247.50$ Hz)	138.20	126.46[b]	128.30	122.27
C-5‴	128.81	131.25	128.96	115.68 (d, $^2J_{C\text{-}F} = 22.13$ Hz)	129.40	127.76[b]	128.79	131.92
C-6‴	126.03	127.61	127.25	127.73 (d, $^3J_{C\text{-}F} = 9.05$ Hz)	125.90	128.44[b]	126.03	127.57
C-7‴	–	–	–	–	–	128.44[b]	–	–
C-8‴	–	–	–	–	–	126.22[b]	–	–
C-9‴	–	–	–	–	–	133.22[b]	–	–
C-10‴	–	–	–	–	–	133.56[b]	–	–
CH_3-4‴	–	–	–	–	21.30	–	–	–

[a] For compounds **3a**, **3c–f** and **3g–h**, $CDCl_3$ was used as a solvent and in case of **3b,** mixture of $CDCl_3$ and trifluoroacetic acid (TFA)

[b] Exchangeable

amphotericin-B, when Ar is naphthyl in comparison with substituted phenyl.

- High inhibitory potential was observed in case of the presence of 4-methylphenyl substituent on thiazole moiety in comparison with phenyl and 4-halophenyl groups.
- In conclusion, compounds **3g–l** may serve as an excellent class of antifungal agents in future, especially *C. albicans*-related problems.

Scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

Antioxidant potential of all synthesized was studied in the concentration range of 50–400 μg/mL using DPPH assay. It has been found that none was found active in scavenging DPPH radical.

Effects of compounds on plasmid DNA under UV irradiation

The effects of compounds **3a–p** on plasmid DNA under UV irradiation were studied using agarose gel electrophoresis method (Fig. 4).

It has been reported in the literature that when the single-strand breaking occurs, the supercoiled (SC) form changes into the more relaxed open circular (OC) form and, however, when breaking of double strand occurs, the SC form transforms into the linear (LC) form. In the present investigation, it has been observed that in the absence of UV irradiation, plasmid DNA existed in SC form (lane 1), but on exposure to UV light most of it was transformed into OC form along with a very less intense LC form as shown in lane 2.

The potential of the test compounds was assessed by comparing the bands appeared in control (**C**) and test compounds in the presence of UV irradiation.

Table 2 ^{13}C NMR data of the synthesized compounds **3i–p**

Compounds[a]	**3i**	**3j**	**3k**	**3l**	**3m**	**3n**	**3o**	**3p**
C-3	145.37	145.29	145.15	145.35	145.19	145.34	145.31	145.23
CH_3-3	14.74	14.75	14.77	14.75	14.74	14.73	14.73	14.72
C-4	137.25	137.22	137.19	137.26	137.05	137.09	137.07	137.03
C-5	141.70	141.68	141.76	141.78	141.76	141.69	141.67	141.64
CH_3-3	12.03	12.03	12.06	12.11	12.03	12.02	12.01	12.00
C-1′	153.35	153.35	153.40	153.41	149.92 (d, $^4J_{C\text{-}F}$ = 3.02 Hz)	149.88 (d, $^4J_{C\text{-}F}$ = 3.02 Hz)	149.87 (d, $^4J_{C\text{-}F}$ = 3.02 Hz)	149.86 (d, $^4J_{C\text{-}F}$ = 3.02 Hz)
C-2′	122.12	122.12	122.12	122.12	123.91 (d, $^3J_{C\text{-}F}$ = 8.05 Hz)	123.93 (d, $^3J_{C\text{-}F}$ = 8.05 Hz)	123.93 (d, $^3J_{C\text{-}F}$ = 9.05 Hz)	123.91 (d, $^3J_{C\text{-}F}$ = 9.05 Hz)
C-3′	129.00	128.99	128.99	129.02	115.90 (d, $^2J_{C\text{-}F}$ = 23.14 Hz)	115.91 (d, $^2J_{C\text{-}F}$ = 23.14 Hz)	115.91 (d, $^2J_{C\text{-}F}$ = 23.14 Hz)	115.88 (d, $^2J_{C\text{-}F}$ = 23.14 Hz)
C-4′	130.14	130.11	130.05	130.11	163.82 (d, $^1J_{C\text{-}F}$ = 250.52 Hz)	163.86 (d, $^1J_{C\text{-}F}$ = 250.52 Hz)	163.85 (d, $^1J_{C\text{-}F}$ = 250.52 Hz)	163.83 (d, $^1J_{C\text{-}F}$ = 250.52 Hz)
C-5′	129.00	128.99	128.99	129.02	115.90 (d, $^2J_{C\text{-}F}$ = 23.14 Hz)	115.91 (d, $^2J_{C\text{-}F}$ = 23.14 Hz)	115.91 (d, $^2J_{C\text{-}F}$ = 23.14 Hz)	115.88 (d, $^2J_{C\text{-}F}$ = 23.14 Hz)
C-6′	122.12	122.12	122.12	122.12	123.91 (d, $^3J_{C\text{-}F}$ = 8.05 Hz)	123.93 (d, $^3J_{C\text{-}F}$ = 8.05 Hz)	123.93 (d, $^3J_{C\text{-}F}$ = 9.05 Hz)	123.91 (d, $^3J_{C\text{-}F}$ = 9.05 Hz)
CH_3-4′	–	–	–	–	–	–	–	–
C-2″	161.81	161.74	161.45	161.70	161.57	161.82	161.79	161.71
C-4″	151.62	151.76	152.88	152.83	152.84	151.70	151.66	151.79
C-5″	109.44	108.63	108.30	109.61	109.19	109.62	109.49	108.66
C-1‴	132.61	130.42 (d, $^4J_{C\text{-}F}$ = 4.02 Hz)	131.48	125.12[b]	134.14	133.04	132.60	130.39 (d, $^4J_{C\text{-}F}$ = 3.02 Hz)
C-2‴	127.25	127.73 (d, $^3J_{C\text{-}F}$ = 8.05 Hz)	125.93	131.49[b]	126.02	127.55	127.25	127.72 (d, $^3J_{C\text{-}F}$ = 8.05 Hz)
C-3‴	128.95	115.70 (d, $^2J_{C\text{-}F}$ = 21.13 Hz)	129.45	123.89[b]	128.80	131.92	128.96	115.70 (d, $^2J_{C\text{-}F}$ = 22.13 Hz)
C-4‴	134.04	162.77 (d, $^1J_{C\text{-}F}$ = 248.51 Hz)	138.15	126.46[b]	128.33	122.29	134.08	162.78 (d, $^1J_{C\text{-}F}$ = 248.51 Hz)
C-5‴	128.95	115.70 (d, $^2J_{C\text{-}F}$ = 21.13 Hz)	129.45	127.76[b]	128.80	131.92	128.96	115.70 (d, $^2J_{C\text{-}F}$ = 22.13 Hz)
C-6‴	127.25	127.73 (d, $^3J_{C\text{-}F}$ = 8.05 Hz)	125.93	128.47[b]	126.02	127.55	127.25	127.72 (d, $^3J_{C\text{-}F}$ = 8.05 Hz)
C-7‴	–	–	–	128.45[b]	–	–	–	–
C-8‴	–	–	–	126.27[b]	–	–	–	–
C-9‴	–	–	–	133.24[b]	–	–	–	–
C-10‴	–	–	–	133.57[b]	–	–	–	–
CH_3-4‴	–	–	21.34	–	–	–	–	–

[a] For compounds **3i–l** and **3m–p**, $CDCl_3$ was used as a solvent

[b] Exchangeable

Among **3a–f**, the compounds **3d** and **3e** protected the DNA from UV damaging effects as observed from the prevention of the DNA degradation by preserving initial supercoiled conformation in comparison with control, while **3a**, **3c** and **3f** compounds were found to behave like control (**C**). However, in **3b**, the intensity of SC was completely diminished and OC form was reduced in comparison with control, which indicated that it exhibited partial photocleaving effect. In case of **3g–l**, the compound **3j** exhibited very high protective effect as indicated by an appearance of high-intensity SC form along with a less intense OC form, in comparison with **3g**, **3i** and **3k** that displayed moderate protective effects. The compound **3l** showed less protective and more photocleaving effect as indicated by less intense SC as well as OC bands, which may be due to degradation of these forms into smaller

Scheme 2 Reaction of *(E)*-3,5-dimethyl-4-(*p*-tolyldiazenyl)-1*H*-pyrazole-1-carbothioamide **1a** with phenacyl bromide **2a** under solvent-mediated conditions

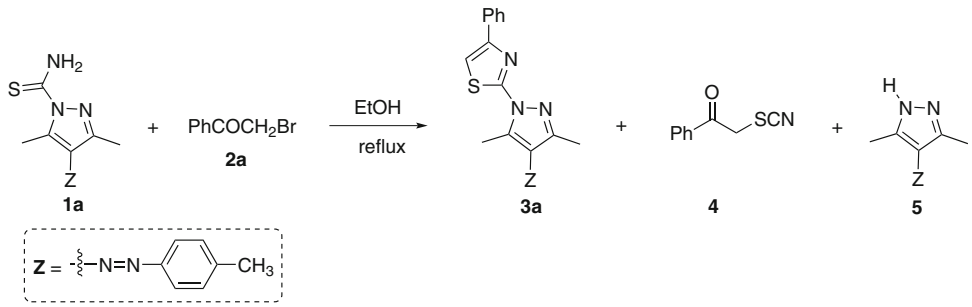


Table 3 Inhibition zone diameter of **3a**, **3b**, **3d** and **3g–p** (in mm) using agar well diffusion method at 400 μg/100 μL

Compound	**3a**	**3b**	**3d**	**3f**	**3g**	**3h**	**3i**	**3j**	**3k**	**3l**	**3m**	**3n**	**3o**	**3p**	Standard
IZ[a] against *E. coli*	12	12	12	–	–	–	–	–	–	–	–	–	–	–	25.0 (ciprofloxacin)
IZ[a] against *C. albicans*	12	14	13	12	24	21	22	24	25	50	12	13	13	12	16.6 (amphotericin-B)

None of the compounds produce inhibition zone against Gram-positive bacterial strains, Gram-negative strain, viz. *P. aeruginosa*, and yeast strain, viz. *S. cerevisiae*

– no activity

[a] Values, including diameter of the well (8 mm), are means of three replicates

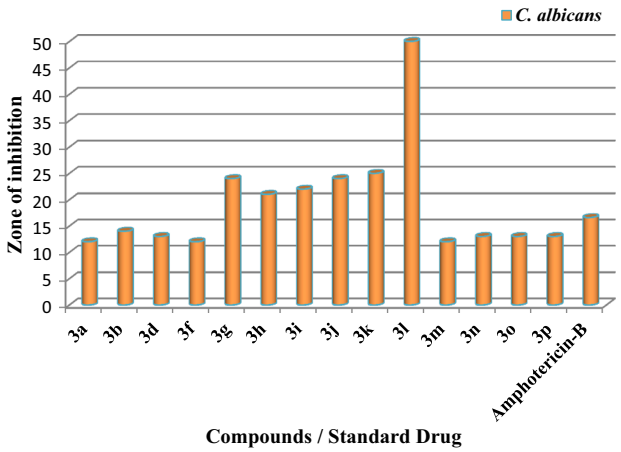


Fig. 3 Comparison of zones of inhibition (in mm) of **3a**, **3b**, **3d** and **3g–p** in reference to amphotericin-B (antifungal drug)

pieces. On the other hand, in case of **3m–p**, the compounds **3m** and **3o** protected the DNA from UV radiation. On the other hand, **3n** and **3p** displayed opposite effects. In compound **3n**, the intensity of OC form was decreased in comparison with control (**C**), which may be due to degradation of OC form, while in **3p** along with decrease in intensity of OC form, increase in intensity of LC form was also observed. In this investigation, among all compounds, **3j** and **3n** were found to be the most potent DNA damage-protecting and DNA photocleavage agents, respectively.

Some important points drawn from the study are given below.

- In case of compounds having R = CH_3, more DNA damage-protecting effects were observed, when *p*-fluorophenyl and *p*-methylphenyl groups are attached to the thiazole moiety. However, partial photocleavage activity was observed in case of the compounds bearing *p*-bromophenyl group. No protective as well as photo-cleaving effects were observed when phenyl, *p*-chlorophenyl and 2-naphthyl groups are linked with the thiazole ring.
- In case of compounds with R = H, increase in DNA damage-protecting effect was observed in the presence of *p*-fluorophenyl moiety attached to thiazole nucleus, while phenyl, *p*-chlorophenyl and *p*-methylphenyl groups were found to be responsible to show moderate protective effects. Lesser protective and more photo-cleaving effect was observed with the compound bearing 2-naphthyl moiety.

Table 4 Antimicrobial activity (expressed as MIC) of compounds **3g–l** against *C. albicans*

Compounds	**3g**	**3h**	**3i**	**3j**	**3k**	**3l**	Amphotericin-B
MIC (in μg/100 μL)	25	25	25	25	12.5	6.25	**12.5**

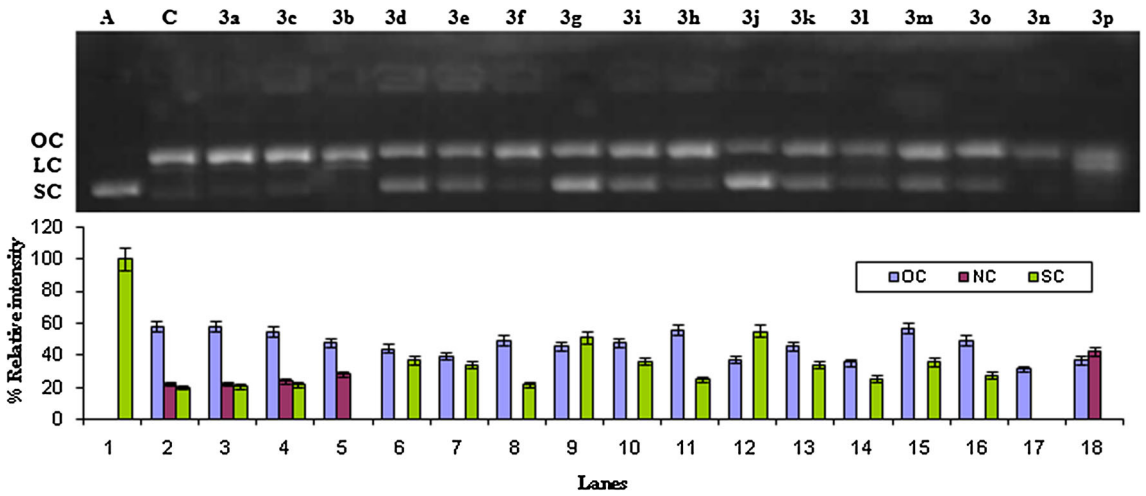
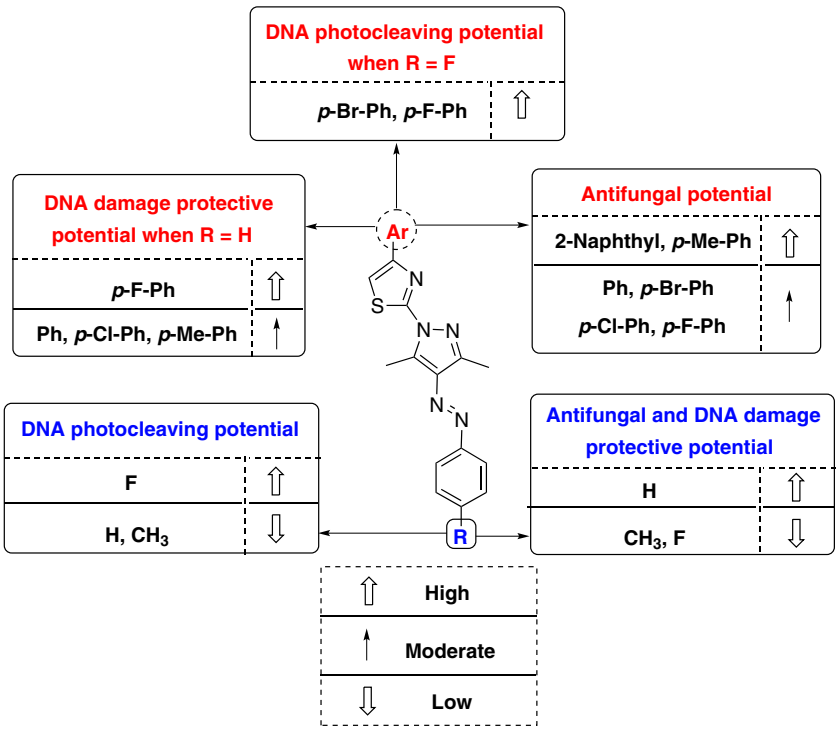


Fig. 4 Effects of compounds **3a–p** on plasmid DNA under UV irradiation: *lane 1* DNA + DMSO without UV, *lane 2* DNA + DMSO + UV, *lane 3* DNA + **3a** + UV, *lane 4* DNA + **3c** + UV, *lane 5* DNA + **3b** + UV, *lane 6* DNA + **3d** + UV, *lane 7* DNA + **3e** + UV, *lane 8* DNA + **3f** + UV, *lane 9* DNA + **3g** + UV, *lane 10* DNA + **3i** + UV, *lane 11* DNA + **3h** + UV, *lane 12* DNA + **3j** + UV, *lane 13* DNA + **3k** + UV, *lane 14* DNA + **3l** + UV, *lane 15* DNA + **3m** + UV, *lane 16* DNA + **3o** + UV, *lane 17* DNA + **3n** + UV, *lane 14* DNA + **3p** + UV

Fig. 5 Substitution pattern/bioactivity



- In compounds bearing R = F, DNA damage-protecting effect was observed when Ar is phenyl and *p*-chlorophenyl. However, *p*-bromophenyl and *p*-fluorophenyl moieties were found to be associated with significant DNA-photocleaving effects.

The overall effects of substitution pattern on the biological activities are summarized in Fig. 5.

Conclusion

In conclusion, a series of sixteen novel (*E*)-2-(3,5-dimethyl-4-(aryldiazenyl)-1*H*-pyrazol-1-yl)-4-arylthiazoles **3a–p** have been synthesized under mild and greener reaction conditions. The structures of the compounds were established on the basis of rigorous analysis of their IR, NMR

(^{1}H and ^{13}C), COSY, ROSEY, HSQC and HMBC spectral data as well as elemental percentages. A structure–activity relationship revealed that the compounds bearing aryl groups at position-4 of the thiazole nucleus in the presence of unsubstituted arylazo moiety (R = H) attached to position-4 of the pyrazole ring were emerged as potent antifungal agents which possessed very high inhibitory action selectively against *C. albicans,* a yeast strain. The compound **3l** was found to be the most active agent even higher than the reference antifungal drug. The compound **3k** bearing R = H and Ar = *p*-methylphenyl exhibited inhibitory potency similar to the standard drug against *C. albicans,* while compounds **3g–j** having R = H and Ar = phenyl and 4-halophenyl exhibited moderate inhibitory potency against *C. albicans*, which was 50 % of the standard drug. Therefore, the compounds **3l** and **3k** could serve as new lead to act as antifungal agents in future. In DNA-based study, the compounds **3j** and **3n** were found as the most potent DNA damage-protecting and DNA photocleavage agents, respectively. Therefore, the compound **3j** may act as a template for the synthesis of newer potent DNA-protecting agents and can be used as potent anti-UV agents, while **3k** may provide the skeleton for synthesis of newer and potent DNA-photocleaving agents which may serve as potent anticancer/antitumor agents in the future.

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Compliance with ethical standards

Conflict of interest None.

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