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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Antimicrobial, analgesic, DPPH scavenging activities and molecular docking study of some 1,3,5-triaryl-2-pyrazolines

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Abstract A series of 1,3,5-triaryl-2-pyrazolines **2a–g** were synthesized by the reaction of 4,4′-disubstituted chalcone with phenyl hydrazine. All these compounds were characterized by NMR, IR and mass spectral and single crystal XRD data. All the synthesized products were screened for their in vitro antimicrobial, analgesic and antioxidant properties. The docking studies were carried out for these compounds against the active site of methionyl-tRNA synthetase (metRS). Some of the tested compounds exhibited significant antimicrobial, analgesic, DPPH scavenging activities and molecular binding.

Keywords Analgesic · Antimicrobial · DPPH scavenging assay · 1,3,5-Triaryl-2-pyrazoline · Molecular docking · metRS · Molecular recognition

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Introduction

Pyrazolines are well-known, and important nitrogen-containing five-membered heterocyclic compounds and various methods have been reported for their synthesis (Fustero *et al.,* 2009; Safaei-Ghomi *et al.,* 2006; Rajendra Prasad *et al.,* 2005). Substituted pyrazolines are useful in pharmaceutical and agrochemical research. They display various biological activities such as antitumor, antibacterial, antifungal, antiviral, antiparasitic, anti-tubercular and insecticidal (Amir *et al.,* 2008; Hes *et al.,* 1978; Grosscurt *et al.,* 1979). Some of these compounds have also antioxidant, anti-inflammatory and analgesic properties (Sarojini *et al.,* 2010; Amir and Kumar, 2005). Owing to these interesting activities of diversely substituted pyrazolines as biological agents considerable attention has been focused on this class. Several 1,3,5-triaryl-2-pyrazolines were also used as scintillation solutes (Wiley *et al.,* 1958). Pyrazoline derivatives with a phenyl group at the 5-position have possessed good film-forming properties, exhibit excellent characteristics of blue photoluminescence, fluorescence and electroluminescence (Zhang *et al.,* 2000). In addition, pyrazolines have played a crucial part in the development of theory in heterocyclic chemistry and also used extensively in organic synthesis (Klimova *et al.,* 1999).

According to oxidative and nitrosylative damage hypothesis, reactive oxygen species (ROS) and reactive nitrogen species (RNS) play important roles in the initiation and promotion of neurodegeneration in the brains of patients with Alzheimer's disease (AD). Some of these free radicals are released during inflammatory reactions, whereas others are formed during normal oxidative metabolism and auto-oxidation of certain neurotransmitters and by β-amyloid. Thus, the role of free radicals in

the pathogenesis of AD should be considered, at least in part, independent of inflammatory reactions. Clinical studies showing the beneficial effects of high-dose antioxidants such as vitamin E and nicotinamide adenine dinucleotide (NADH) in the treatment of AD support the role of free radicals in progressive degeneration of neurons. Edaravone "(3-methyl-1-phenyl-2-pyrazolin-5-one)", a strong novel free radical scavenger, was used for treatment of patients with acute brain infarction. Antioxidant actions of edaravone include enhancement of prostacyclin production, inhibition of lipoxygenase metabolism of arachidonic acid by trapping hydroxyl radicals, inhibition of alloxan-induced lipid peroxidation, and quenching of active oxygen, leading to protection of various cells, such as endothelial cells, against damage by ROS (Kokura *et al.,* 2005).

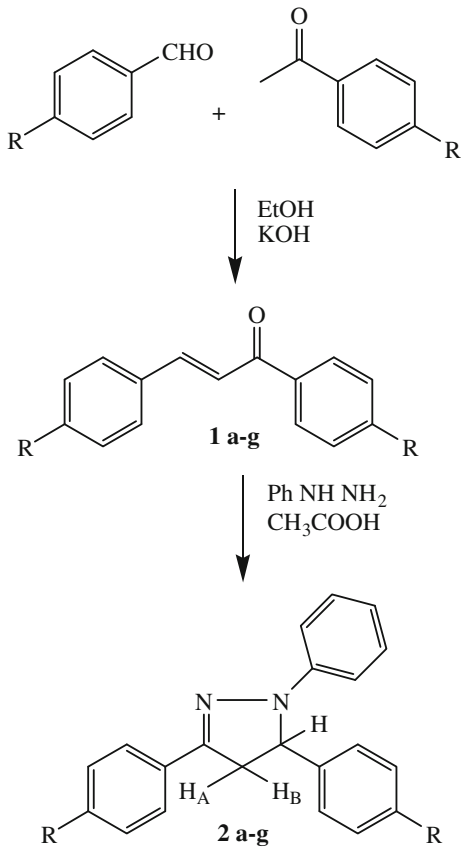
There is an urgent need to develop novel classes of antibiotics to counter the inexorable rise of resistant bacterial pathogens. Modern antibacterial drug discovery is focused on the identification and validation of novel protein targets that may have a suitable therapeutic index. In combination with assays for function, the advent of microbial genomics has been invaluable in identifying novel antibacterial drug targets. The major challenge in this field is the implementation of methods that validate protein targets leading to the discovery of new chemical entities. Ligand-directed drug discovery has the distinct advantage of having a concurrent analysis of both the importance of a target in the disease process and its amenability to functional modulation by small molecules. VITA^{TM} is a process that enables a target-based paradigm by using peptide ligands for direct in vitro and in vivo validation of antibacterial targets and the implementation of high-throughput assays to identify novel inhibitory molecules. This process can establish sufficient levels of confidence indicating that the target is relevant to the disease process and inhibition of the target will lead to effective disease treatment (Lapan *et al.*, 2002).

As evident from the literature, in recent years a significant portion of research work in heterocyclic chemistry has been devoted to 2-pyrazolines containing different aryl groups as substituents. Among the methods employed in synthesis of pyrazolines, condensation of substituted chalcones with hydrazine and its derivatives is commonly used (Knorr, 1893; Thakare and Wadodkar, 1986; Ankhiwala and Hathi, 1996; Azarifar and Ghasemnejad, 2003). In view of the importance of 2-pyrazolines and in continuation of our work on pyrazolines (Samshuddin *et al.*, 2010; Jasinski *et al.*, 2010a, b; Fun *et al.*, 2010; Baktir *et al.*, 2011), we report the synthesis and biological evaluation of some 1,3,5-triaryl-2-pyrazolines.

Results and discussion

Chemistry

A series of 1,3,5-triaryl-2-pyrazolines **2a–g** were synthesized by the reaction of 4,4′-disubstituted chalcone with phenyl hydrazine (Scheme 1). The structures of title compounds were confirmed by ^{1}H and ^{13}C NMR, IR, LCMS and elemental analysis. The structure of compounds **2a**, **2c**, **2e** and **2f** were characterized by single crystal XRD [**2a**: Monoclinic; $P2_1/c$; $a = 12.2880\ (3)$ Å; $b = 13.1678\ (3)$ Å; $c = 11.3245\ (3)$ Å; $V = 1690.91\ (7)$ Å$^{3;}$ $Z = 4$; **2c**: Orthorhombic; $P2_12_12_1$;$a = 10.5815\ (3)$ Å; $b = 11.2119\ (3)$ Å; $c = 15.4569\ (4)$ Å; $V = 1833.79\ (9)$ Å^3; $Z = 4$; **2e**: Monoclinic, $P2_1/n$; $a = 5.8113\ (3)$ Å; $b = 10.6959\ (5)$ Å;$c = 28.4455\ (13)$ Å;$V = 1761.41\ (15)$ Å$^{3;}$ $Z = 4$; **2f:** Monoclinic; $P2_1/a$; $a = 9.4788\ (5)$ Å; $b = 10.1893\ (6)$ Å; $c = 19.9139\ (10)$ Å; $V = 1921.79\ (18)$ Å$^{3;}$ $Z = 4$] (Fig. 1) (Samshuddin *et al.*, 2010; Jasinski *et al.*, 2010a, b; Baktir *et al.*, 2011; Butcher *et al.*, 2011). The IR spectra of all the compounds showed –C=N– stretch at 1580–1595 cm^{-1} confirmed the formation of pyrazoline moiety. In the ^{1}H NMR spectra of pyrazoline, protons H_A and H_B are geminal



Scheme 1 Synthesis of 1,3,5-triaryl-2-pyrazolines **2a–g**

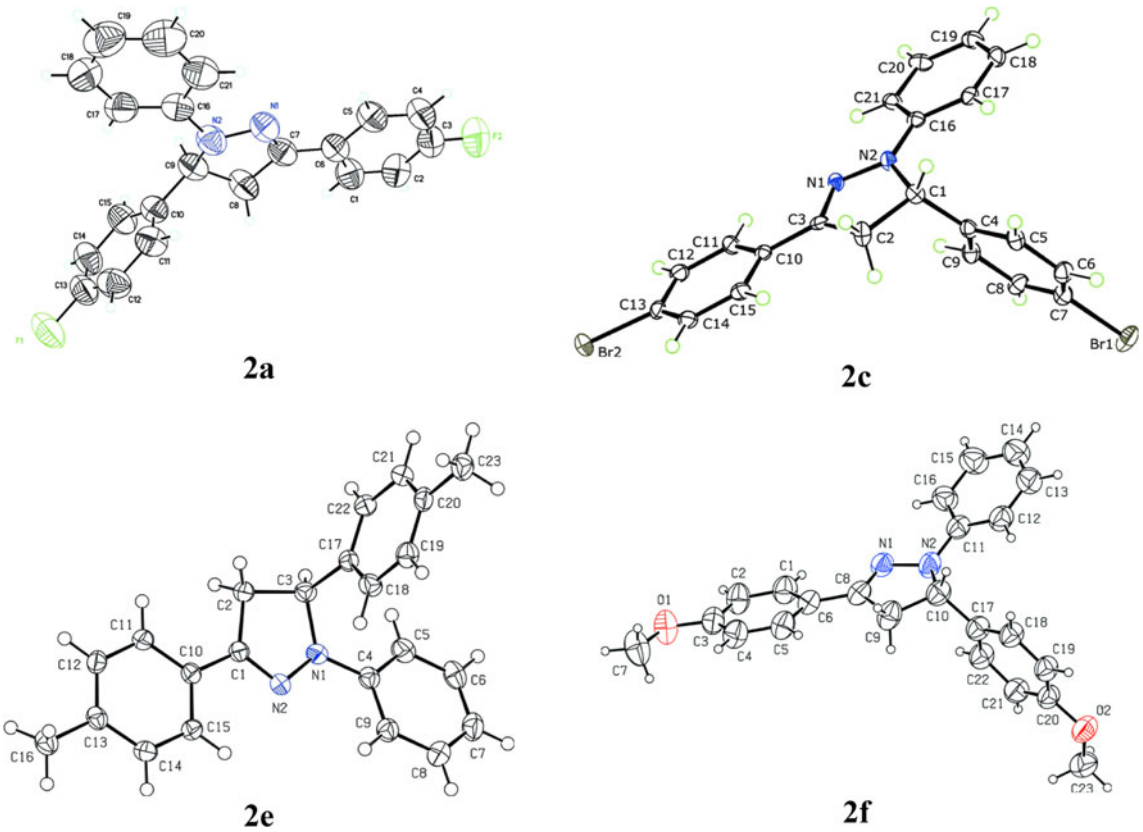


Fig. 1 The molecular structure and numbering scheme for the compounds **2a**, **2c**, **2e**, and **2f**, with displacement ellipsoids drawn at the 50% probability level for **2a**, **2c**, and **2e** and 30% probability level for **2f**

protons at C4 carbon, appeared in the region 3.00–3.14 ppm ($J = 6.3$ Hz) and 3.80–3.98 ppm ($J = 12.3$ Hz) as doublet of doublets for all newly synthesized compounds. The CH proton at C5 also appeared as doublet of doublets in the region of 5.37–5.57 ppm ($J = 6.3$ Hz), due to vicinal coupling with two non-equivalent geminal protons of C4 carbon. LCMS and ^{13}C-NMR spectral data supported the formation of **2a**–**g**. Elemental analysis also gave satisfactory results for all the compounds.

Biological evaluation

Antimicrobial studies

The synthesized 1,3,5-triaryl-2-pyrazolines **2a**–**g**, were assayed for their antimicrobial activities against four bacterial strains Gram positive, *Bacillus subtilis, Streptococcus haemolytius;* Gram negative, *Pseudomonas aeruginosa, Klebsiella pneumoniae.* The compounds were also tested against two fungal strains *Aspergillus niger*, *Candida albicans* using agar well diffusion method (Sharath *et al.*, 2008; Saundane *et al.*, 1998; Raghavendra and Neelagund, 2009). Further, their MIC values were determined against these organisms by micro dilution method (Raghavendra and Neelagund, 2009; Harish *et al.*, 2007) using DMSO as a solvent. Ciprofloxacin and Fluconazole were used as standard antibiotics. All the tested compounds were emerged as active against all tested microorganisms.

The different substitutions on the pyrazoline moiety almost equally contribute to the antimicrobial activity comparable with that of standard drugs tested. However, based on this promising observation, it is immature to arrive at the conclusion on structure activity relationship aspect of these molecules and further evaluation is needed to use them for clinical use.

Analgesic activity

The analgesic activity of compounds **2a**–**g** was performed by the acetic acid-induced writhing test in mice (Vagdevi *et al.*, 2001; Bagavant *et al.*, 1994; Satyanarayana and Rao 1993). Among the tested compounds, 3,5-bis (4-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazole **2f**,

3,5-bis(4-methylphenyl)-1-phenyl-4,5-dihydro-1H-pyrazole **2e** and 1,3,5-triphenyl-4,5-dihydro-1H-pyrazole **2d** exhibited good analgesic activity compared with acetyl salicylic acid as a standard analgesic agent, whereas all other compounds showed moderate activity. The enhanced activity of these compounds might be attributed to methoxy phenyl, methylphenyl and phenyl groups present in the pyrazoline molecules.

DPPH radical scavenging assay

A rapid, simple and inexpensive method to measure antioxidant capacity of substances involves the use of the free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free-radical scavengers or hydrogen donors. Antioxidants tested on DPPH were also found extremely effective in cell systems. This simple test further provides information on the ability of a compound to donate electrons during antioxidant action (Tiwari, 2004). The radical scavenging mechanism is based on the transfer of H-atom from the methylene group of 1,3,5-triaryl-2-pyrazolines to DPPH radical to form DPPH-H. Among the tested compounds, 5-bis(4-flourophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazole **2a** showed good radical scavenging capacity whereas 5-bis(4-bromophenyl)-1-phenyl-4, 5-dihydro-1H-pyrazole **2c** and 5-bis(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazole **2f** exhibited moderate radical scavenging capacity with concentration of 10 μg/ml in comparison with the standard ascorbic acid. All other compounds showed low activity. The variation exhibited in DPPH scavenging capacity could be attributed to the effect of different substitutions.

Docking calculations with ICM^{TM} (Internal coordinate mechanics) dock

Molecular docking and virtual screening based on molecular docking have become an integral part of many modern structure-based drug discovery efforts. The binding of small molecule ligands to large protein targets is central to numerous biological processes. The accurate prediction of the binding modes between the ligand and protein (the docking problem) is of fundamental importance in modern structure-based drug design (Taylor *et al.*, 2002). Molecular docking for the screening of anti-SARS drugs (Wei *et al.*, 2006) and the docking studies of antityrosinase activity of the Thai mango seed kernel extract are just a few good examples of docking studies that have shown a better results for further analysis (Nithitanakool *et al.,* 2009). So an *in silico* docking of the newly synthesized compounds to methionyl-tRNA synthetase enzyme was attempted. Many tRNA synthetases can be considered good targets for antibacterial discovery because they are broadly conserved, essential for growth and distinct enough from their human orthologs to anticipate the discovery of selective inhibitors (Hurdle *et al.*, 2005).

Putative molecular interactions with metRS

The compounds **2a**–**g** have been docked into the active site of the active site of methionyl-tRNA synthetase (metRS) (PDB ID: 1A8H).

Oxygen atom of the Glu138 formed a hydrogen bond (2.73 Å) with F1 atom of compound **2a** and N1 atom of the compound formed the second hydrogen bond with the H21 atom of Arg132 with a length of 2.5 Å. All the phenyl rings of the same compound formed hydrophobic interactions and π–π stacking with different atoms and phenyl rings of different amino acid residues like, Glu138, Tyr134, Thr55, Ile146 and Pro145.

Compound **2b** showed one hydrogen bonding between the H atom of Tyr134 and N1 atom of the compound with a length of 2.46 Å. Similar like compound **2a** this one also exhibited several hydrophobic interactions with Pro145, Ile146, Thr55, Arg132 and Glu54.

Very similar sorts of interactions have been observed in case of compound **2c** like **2a** and **2b**. The same N1 atom of compound **2c** formed hydrogen bond between H-atom of Tyr134 with a length of 2.5 Å and other phenyl rings exhibited similar hydrophobic interactions and π–π stackings.

Very similar interactions have been observed also by compounds **2d**, **2e** and **2f**. Compound **2g** also showed similar interaction, difference only is instead of Tyr134 the N1 atom of the compounds formed hydrogen bonding with HE2 atom of His147 residue and hydrophobic interactions with Arg149, His147, Ser129, Ile146 and Thr55.

All the molecules exhibited comparable binding energy varying from −2.2 to −4.1 kcal/mol. But the docking energy varied from −4.2 o −85.6 kcal/mol. Docking energy gives an idea about the energy required to cover the entire protein by a ligand molecule whereas the binding energy gives the information about the putative interaction of the molecules at the active site of the enzyme. Lower the value of these energies, efficient will be the molecule. So this study indicates that the compound **2g** is most active among the docked compounds. However, both in vitro and *in silico* studies complement each other. Further in vivo studies are needed to know the exact nature of the compounds to recommend them as possible drug candidates.

Experimental section

Chemistry

Melting points were taken in open capillary tubes and are uncorrected. The purity of the compounds confirmed by thin layer chromatography using Merck silica gel 60 F_{254} coated aluminium plates. IR spectra were recorded on Shimadzu-FTIR Infrared spectrometer in KBr (ν_{max} in cm^{-1}). ^1H(400 MHz) NMR spectra were recorded on a Bruker AMX 400 or Bruker DPX 300 spectrometer, with 5 mm PABBO BB -1H TUBES and ^{13}C (100 MHz) NMR spectra were recorded for approximately 0.03 M solutions in DMSO-d6 at 75 MHz or 100 MHz with TMS as internal standard. LCMS were obtained using Agilent 1200 series LC and Micromass zQ spectrometer. Elemental analysis was carried out by using VARIO EL-III (Elementar Analysensysteme GmBH). All chemicals were purchased from Sigma-Aldrich Co., U.S.A.

General procedure for synthesis of 1,3,5-triaryl-2-pyrazolines (**2a–g**)

A series of 4,4′-disubstituted chalcones **1a–g** were prepared by stirring a mixture of *p*-substituted benzaldehyde (0.02 mol) and *p*-substituted acetophenone (0.02 mol) in ethanolic potassium hydroxide solution (50 ml) for several hours at 5–10°C. The precipitate thus formed was collected by filtration and purified by recrystallization from ethanol. Further, a solution of each of these chalcones **1a–g** (0.01 mol) and phenyl hydrazine (0.01 mol) in 50 ml glacial acetic acid was refluxed for 6 h. The reaction mixture was cooled and poured onto 50 ml ice-cold water. The precipitate was collected by filtration, dried and re-crystallized from ethanol or acetonitrile to obtain the pure 1,3,5-triaryl-2-pyrazolines **2a–g**. Characterization data are given in Table 1.

*3,5-Bis(4-flourophenyl)-1-phenyl-4,5-dihydro-1H-pyrazole **(2a)***

^1H NMR (DMSO, 400 MHz): δ 3.08–3.14(dd, 1H, CH_2-H_A, $J = 6.36$ Hz), 3.86–3.94(dd, 1H, CH_2–H_B, $J = 12.24$), 5.48–5.53(dd, 1H, Ar–CH, 6.32 Hz), 6.70–7.78(m, 13H, Ar–H). LCMS: *m/z* 335.4 (M^++1). IR (KBr, ν cm^{-1}): 3027 (Ar–H), 2926(CH_2), 1587 (Pyrazoline C=N), 1498 (Ar–H).

*3,5-Bis(4-chlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazole **(2b)***

^1H NMR (DMSO, 400 MHz): δ 3.08–3.14(dd, 1H, CH_2–H_A, $J = 6.28$ Hz), 3.86–3.94(dd, 1H, CH_2–H_B, $J = 12.32$), 5.52–5.57(dd, 1H, pyrazoline 5C–H, 6.28 Hz), 6.72–7.74(m, 13H, Ar–H). LCMS: *m/z* 367.3 (M^+), 365.3 (M^+-2). IR (KBr, ν cm^{-1}): 3032 (Ar–H), 2920(CH_2), 1592 (Pyrazoline C=N), 709 (C–Cl), 1503 (Ar–H).

*3,5-Bis(4-bromophenyl)-1-phenyl-4,5-dihydro-1H-pyrazole **(2c)***

^1H NMR (DMSO, 400 MHz): δ 3.08–3.14(dd, 1H, CH_2–H_A, $J = 6.28$ Hz), 3.86–3.94(dd, 1H, CH_2–H_B, $J = 12.32$), 5.50–5.55(dd, 1H, pyrazoline 5C–H, 6.28 Hz), 6.72–7.69(m, 13H, Ar–H). LCMS: *m/z* 456.3 (M^+). IR (KBr,ν cm^{-1}): 3058 (Ar–H), 2922(CH_2), 1580 (Pyrazoline C=N), 528 (C–Br), 1500 (Ar–H).

*1,3,5-Triphenyl-4,5-dihydro-1H-pyrazole **(2d)***

^1H NMR (DMSO, 400 MHz): δ 3.08–3.14(dd, 1H, CH_2–H_A, $J = 6.4$ Hz), 3.88–3.96(dd, 1H, CH_2–H_B, $J = 12.28$), 5.45–5.50(dd, 1H, pyrazoline 5C–H, 6.4 Hz), 6.69–7.74(m, 15H, Ar–H). ^{13}C NMR (100 MHz, DMSO): δ 43.05, 63.21, 113.00, 118.67, 125.75, 125.91, 127.46, 128.70, 128.75, 128.92, 129.09, 132.34, 142.62, 144.30, 147.24. LCMS:

Table 1 Characterization data of 1,3,5-triaryl-2-pyrazolines **2a–g**

Compounds	Molecular formula	*R*	Yield (%)	Melting point (°C)	Elemental analysis %, found (calculated)		
					C	H	N
2a	$C_{21}H_{16}F_2N_2$	–F	84	112–114	75.40 (75.43)	4.77 (4.82)	8.31 (8.38)
2b	$C_{21}H_{16}Cl_2N_2$	–Cl	77	120–122	68.59 (68.68)	4.32 (4.39)	7.55 (7.63)
2c	$C_{21}H_{16}Br_2\ N_2$	–Br	86	206–208	55.21 (55.29)	3.48 (3.54)	6.10 (6.14)
2d	$C_{21}H_{18}N_2$	–H	79	134–135	84.46 (84.53)	6.03 (6.08)	9.34 (9.36)
2e	$C_{23}H_{22}N_2$	$–CH_3$	78	141–142	84.56 (84.63)	6.71 (6.79)	8.52 (8.58)
2f	$C_{23}H_{22}N_2O_2$	$–OCH_3$	76	139–141	77.01 (77.07)	6.10 (6.19)	7.79 (7.82)
2g	$C_{33}H_{26}N_2$	–Ph	83	136–138	87.91 (87.97)	5.77 (5.82)	6.16 (6.22)

m/z 297.4 (M^+-1). IR (KBr,ν cm^{-1}): 3020 (Ar–H), 2921(CH_2), 1595 (Pyrazoline C=N), 1490 (Ar–H).

3,5-Bis(4-methylphenyl)-1-phenyl-4,5-dihydro-1H-pyrazole ***(2e)***

^{1}H NMR (DMSO, 400 MHz): δ 2.07(s, 3H, –CH_3), 2.24(s, 3H, –CH_3), 3.00–3.07 (dd, 1H, CH_2–H_A, $J = 6.28$ Hz), 3.83–3.90(dd, 1H, CH_2–H_B, $J = 12.16$), 5.37–5.42 (dd, 1H, pyrazoline 5C–H, 6.28 Hz), 6.67–7.64(m, 13H, Ar–H). ^{13}C NMR (100 MHz, DMSO): δ 20.69, 21.00, 43.13, 62.93, 112.94, 118.43, 125.71, 125.83, 128.86, 129.28, 129.57, 129.65, 136.56, 138.33, 139.70, 144.44, 147.33. LCMS: *m/z* 325.3 (M^+-1). IR (KBr,ν cm^{-1}): 3030 (Ar–H), 2900(CH_3), 1595 (Pyrazoline C=N), 1490 (Ar–H).

3,5-Bis(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazole ***(2f)***

^{1}H NMR (DMSO, 400 MHz): δ 3.00–3.07(dd, 1H, CH_2–H_A, $J = 6.28$ Hz), 3.70(s, 3H, OCH_3–), 3.79(s, 3H, OCH_3–), 3.80–3.98(dd, 1H, CH_2–H_B, $J = 12.32$), 5.52–5.57(dd, 1H, pyrazoline 5C–H, 6.28 Hz), 6.72–7.74(m, 13H, Ar–H). LCMS: *m/z* 359.4 (M^+-1). IR (KBr,ν cm^{-1}): 3000 (Ar–H), 2950(CH_2), 2825 (OCH_3–),1585 (Pyrazoline C=N), 1490 (Ar–H).

3,5-Di(biphenyl-4-yl)-1-phenyl-4,5-dihydro-1H-pyrazole ***(2g)***

^{1}H NMR (DMSO, 400 MHz): δ 3.04–3.10(dd, 1H, CH_2–H_A, $J = 6.28$ Hz), 3.84–3.92(dd, 1H, CH_2–H_B, $J = 12.32$), 5.42–5.47(dd, 1H, pyrazoline 5C–H, 6.28 Hz), 6.60–7.92(m, 23H, Ar–H). LCMS: *m/z* 450.0 (M^+). IR (KBr,ν cm^{-1}): 3027 (Ar–H), 2925(CH_2), 1584 (Pyrazoline C=N), 1501 (Ar–H).

Biological evaluation

Antimicrobial activity

The antimicrobial activity of synthesized compounds **2a–g** was carried out using agar well-diffusion method. The bacterial strains were collected from different infectious status of patients who had not administered any antibacterial drugs for at least 2 weeks with the suggestions of an authorized physician, in Kiran diagnostic health centre of Chitradurga, Karnataka state, India. Fungal strains were procured from the culture maintained at National College of Pharmacy Shimoga. The in vitro antimicrobial activity was carried out against 24 h culture of four bacterial strains Gram positive *Bacillus subtilis, Streptococcus haemolytius* Gram negative, *Pseudomonas aeruginosa, Klebsiella pneumoniae.* Two fungal strains were *Aspergillus niger and Candida albicans*. The compounds were tested at 40 μg/ml concentration against both bacterial and fungal strains. DMSO was used as a vehicle. Ciprofloxacin and Fluconazole were used as standard drugs for comparison of antibacterial and antifungal activities, respectively. The zone of inhibition was compared with standard drug after 24 h of incubation at 37°C for antibacterial activity and 72 h at 25°C for antifungal activity. The results are recorded in Table 2.

The MIC of all synthesized compounds **2a–g** was determined by a micro dilution method. The respective clinical strain was spread separately on the medium. The wells were created using a stainless steel sterilized cork borer under aseptic conditions. The synthesized compounds at different concentrations viz. 10, 20, 30, 40 and 50 μg was dissolved, respectively, in 25, 50, 75, 100 and 125 μl of DMSO and later loaded into corresponding wells. The standard drug Ciprofloxacin (40 μg in100 μl and Fluconazole (40 μg in100 μl) were used as standard drugs

Table 2 Anti-microbial activity of synthesized compounds **2a–g**

Zone of inhibition in (mm)						
Compounds	Antibacterial strains				Antifungal strains	
	B. subtilis	*Streptococcus haemolytius*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*A. niger*	*C. albicans*
2a	21	23	20	22	20	20
2b	22	24	21	20	22	21
2c	23	21	22	21	20	20
2d	23	23	22	23	23	21
2e	20	22	23	24	24	22
2f	21	23	24	22	21	20
2g	23	21	22	23	22	21
Ciprofloxacin	24	24	24	24	–	–
Fluconazole	–	–	–	–	24	23
Control (DMSO)	0	0	0	0	0	0

Table 3 Minimum inhibitory concentration (MIC) of synthesized compounds **2a–g**

MIC (μg/μl)						
Compounds	Antibacterial strains				Antifungal Strains	
10–50 (μg)	*B. subtilis*	*Streptococcus haemolytius*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*A. niger*	*C. albicans*
2a	30	30	40	30	40	40
2b	30	30	30	30	30	30
2c	40	40	30	40	40	20
2d	40	40	30	40	30	30
2e	30	20	40	30	40	30
2f	40	40	30	30	30	40
2g	40	40	30	30	40	40
Control DMSO	0	0	0	0	0	0

for comparison of antibacterial and antifungal activities, respectively. The zone of inhibition was compared with standard drug after 24 h of incubation at 37°C for antibacterial activity and 72 h at 25°C for antifungal activity. The results are recorded in Table 3.

Analgesic activity

The analgesic activity was performed by the acetic acid-induced writhing test in mice and approved by the animal ethics committee (Institutional Animal Ethical Committee, IAEC) of the National College of Pharmacy, Harapanahalli. Five groups of six male Swiss albino mice, each 25–35 g of body weight, were used. 0.6% acetic acid (dose = 10 ml/kg of body weight) was injected intraperitoneally. The numbers of writhes were counted for 20 min, after 5 min of injection of acetic acid into each mice. This reading was taken as a control. Next day, same groups of mice were used for evaluating analgesic activity. Each group was administered orally with the suspension of test compound in 0.1% Tween-80 solution at a dose of 100 mg/kg body weight of animal was given 1 h before injection of acetic acid. After 5 min of acetic acid injection, mice were observed for the number of writhes for the duration of 20 min. The mean value for each group was calculated and compared with control. Acetyl salicylic acid was used as a standard for comparison of analgesic activity. Results are recorded in Table 4. Percent protection was calculated using following formula: $(1 - V_c/V_t) \times 100$; where V_t = Mean number of writhing in test animals and V_c = Mean number of writhing in control.

Table 4 Analgesic activity of synthesized compounds **2a–g**

Compounds	Dose (mg/kg)	Mean no. of writhing		% Protection
		Before drug	After drug	
2a	100	31.1 ± 0.40	15.66 ± 0.85	49.65
2b	100	31.0 ± 6.73	15.5 ± 0.44	50.0
2c	100	32.0 ± 0.25	14.8 ± 0.31	53.75
2d	100	31.5 ± 0.44	13.3 ± 0.63	57.8
2e	100	32.5 ± 0.57	13.6 ± 0.57	58.16
2f	100	31.5 ± 0.44	13.0 ± 0.36	58.74
2g	100	31.1 ± 0.48	14.6 ± 0.51	53.1
Standard (Aspirin)	100	32.33 ± 0.57	13.5 ± 0.68	58.25

DPPH radical scavenging assay

The DPPH assay was based on the reported method (Kokura *et al.*, (2005). In brief, the DMSO sample of compounds at 10 μg/ml and it was diluted to 4 ml using distilled water. To this 1 ml of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) solution in methanol was added. The mixed solution was incubated at room temperature for 30 min. The absorbance of stable DPPH was read at 517 nm using UV–visible spectrophotometer and the remaining DPPH was calculated. Ascorbic acid was taken as standard. The free radical scavenging activity was expressed as follows:

$$\text{DPPH scavenging activity } (\%) = \frac{[A_c - A_s]}{[A_c - A_b]} \times 100$$

where A_c was the absorbance of the control, A_s for the sample and A_b for the blank (MeOH). Each sample was assayed at 10 μg/ml and all experiments were carried out in triplicate and the % RSC is shown in Table 5.

Docking calculations with ICM™ (internal coordinate mechanics) dock

All the docking calculations of compounds in this article were performed using the ICM™ docking module with the

Table 5 DPPH radical scavenging assay of synthesized compounds **2a–g**

Compounds	%DPPH scavenging
2a	51.22 ± 1.36
2b	31.76 ± 1.33
2c	40.28 ± 2.04
2d	23.13 ± 2.11
2e	22.37 ± 1.38
2f	39.61 ± 1.68
2g	18.22 ± 1.53
Std (Ascorbic acid)	69.22 ± 1.67

default setup as earlier mentioned (Abagyan *et al.*, 1994; Khan *et al.*, 2009).

Preparations of the inhibitors and target molecules

The 2D structures of the compounds (in mol file formats) have been converted to 3D and energy minimized at the 3D space of ICM environment. The atom types using local chemical environment, Merck molecular force field (MMFF) (Halgren, 1996a, b, c, d, 1999a, b; Halgren and Nachbar1996) formal charges and 3D topology were assigned. The lowest energy conformers of the compounds were then docked into the 3D space of the targets active site [methionyl-tRNA synthetase (metRS) (PDB ID: 1A8H)] (Sugiura *et al.*, 2000).

Docking process

All the docking calculations were performed using the 'interactive docking' menu at the ICM environment. After docking the stack of docking poses were checked visually. Multiple stack conformations were selected based on their docking energies, rmsd values (compared between the docked model and X-ray conformation) and similarities to closely related X-ray crystal structures from PDB. Then the best conformations for each of the compounds were finally chosen, and then their binding energies were calculated using ICM script (briefly described in the following 'Calculations of free energies of binding' section).

The correlation between the activity profiles and the binding energies (Cal. ΔG) are presented in 'Results and discussion' section.

Calculations of free energies of binding

For each of the individual docked complexes the free energies of binding (Cal. ΔG) between the protein and ligand was calculated using ICM script utilizing the Eqs. 1 and 2 (Schapira *et al.*, 1999).

$$\Delta G = \Delta G_{\mathrm{H}} + \Delta G_{\mathrm{EL}} + \Delta G_{\mathrm{S}} + C \quad (1)$$

$$\Delta G_{\mathrm{EL}} = \Delta G_{\mathrm{COUL}} + \Delta G_{\mathrm{DESOLV}} \quad (2)$$

Here ΔG_{H} is the hydrophobic or cavity term, which accounts for the variation of water/non-water interface area. ΔG_{EL} is the electrostatic term composed of coulombic (ΔG_{COUL}) interactions and desolvation ($\Delta G_{\mathrm{DESOLV}}$) of partial charges transferred from an aqueous medium to a protein core environment. ΔG_{S} is the entropic term which results from the decrease in the conformational freedom of functional groups buried upon complexation; and finally the C is a constant accounts for the change of entropy of the system due to the decrease of free molecules concentration (cratic factor), and loss of rotational/translational degrees of freedom (Schapira *et al.*, 1999). The calculated docking and binding (ΔG) energies (in Kcal/mol) of the compounds are shown in Table 6.

Interpretations of intermolecular interactions

To study the intermolecular interactions between the targets and the compounds LigPlot (Wallace *et al.*, 1995) were used to plot the interactions from 3D to 2D. Beside LigPlot, ICM (http://www.molsoft.com) and Discovery Studio Visualizer (http://www.accelrys.com) also been used to analyze the interactions in 3D space. The molecular interactions between the compounds and the active site residues of metRS at 3D space are shown in Fig. 2, different compounds in different panels, accordingly.

Conclusion

A series of 1,3,5-triaryl-2-pyrazolines **2a–g** were synthesized by the reaction of 4,4′-disubstituted chalcones with phenyl hydrazine and screened for their antimicrobial, analgesic and antioxidant properties. All the tested compounds were emerged as active against all tested

Table 6 Calculated docking and binding (ΔG) energies of the compounds **2a–g** against metRS

Compounds	Calculated energies (in Kcal/mol)	
	Docking	Binding (ΔG)
2a	−14.0	−2.3
2b	−6.1	−3.3
2c	−6.1	−3.2
2d	−4.2	−2.2
2e	−7.2	−4.1
2f	−12.3	−3.7
2g	−85.6	−3.7

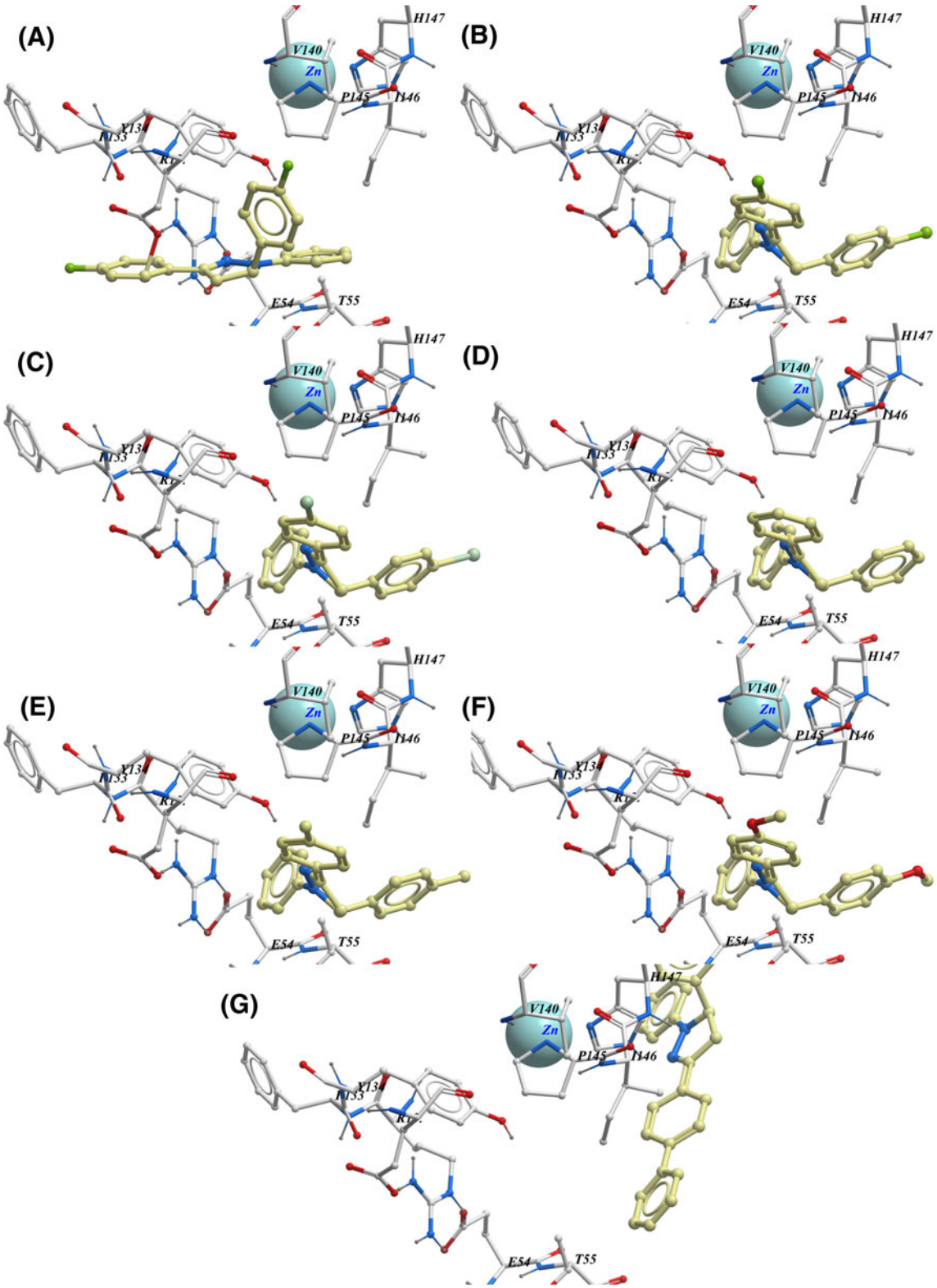


Fig. 2 Molecular interactions between the compounds **2a–g** and the methionyl-tRNA synthetase (metRS) (PDB ID: 1A8H)

microorganisms. Among them, 3,5-bis(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazole **2f**, 3,5-bis(4-methylphenyl)-1-phenyl-4,5-dihydro-1H-pyrazole **2e** and 1,3,5-triphenyl-4,5-dihydro-1H-pyrazole **2d** have exhibited good analgesic activity. The compound 5-bis(4-flourophenyl)-1-phenyl-4,5-dihydro-1H-pyrazole **2a** has shown good

DPPH scavenging activity where as compounds 5-bis (4-bromophenyl)-1-phenyl-4,5-dihydro-1H-pyrazole **2c** and 5-bis(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazole **2f** have shown moderate DPPH scavenging activity. The docking studies are carried out for these compounds against the active site of methionyl-tRNA synthetase (metRS). Some of the tested compounds exhibited good molecular binding. Hence this study has widened the scope of developing these 1,3,5-triaryl-2-pyrazoline derivatives as promising antimicrobial, analgesic and antioxidant agents.

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