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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of some new 3,5-diamino-4-(4′-fluorophenylazo)-1-aryl/heteroarylpyrazoles as antimicrobial agents

Ranjana Aggarwal · Virender Kumar · Girish Kumar Gupta · Vinod Kumar

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Abstract Synthesis of some new 3,5-diamino-4-(4′-fluorophenylazo)-1-aryl/heteroarylpyrazoles (**5a–5i)** was achieved by the treatment of aryl/heteroarylhydrazines **4a–4i** with 2-[(4-fluorophenyl)hydrazono]malononitrile **3** in refluxing ethanol. The structure of the compounds was established on the basis of IR, NMR (^{1}H and ^{13}C) and mass spectral studies. All the nine synthesized compounds were screened for their in vitro antimicrobial activity against two Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*), two Gram-negative bacteria (*Escherichia coli*, *Klebesiella aerogenes*) and three pathogenic fungi (*Aspergillus niger*, *Candida albicans*, and *Saccharomyces cerevisiae*).

Keywords
2-[(4-Fluorophenyl)hydrazono]malononitrile · Aryl/heteroarylhydrazines · 3,5-Diamino-4-(4′-fluorophenylazo)-1-aryl/heteroarylpyrazoles · Antimicrobial activity

Introduction

The pyrazole ring has been widely employed in the investigation of pharmacologically active heterocyclic compounds. Aminopyrazole system, in particular, has received considerable attention in recent years because of its applications in pharmaceutical and agrochemical industries. Aminopyrazole derivatives display different kind of biological activities such as antibacterial (Aggarwal *et al*., 2006; Kumar *et al*., 2005), antitumor (Samanta *et al*., 2006) and act as highly potent agonists of GPR109b (Skinner *et al*., 2009), selective inhibitors of MK2 (Velcicky *et al*., 2010) and p38a MAP kinase (Das *et al*., 2010). Moreover, they have been successfully employed as active synthons in the synthesis of condensed heterocyclic products such as pyrazolo[3,4-*d*]pyrimidines, pyrazolo[1,5-*a*]pyrimidines, pyrazolo[3,4-*b*]pyridines, etc. which exhibit antiproliferative activity (Schenone *et al*., 2004), antimicrobial (Aggarwal *et al*., 2011), antiviral activity (Johns *et al*., 2007) and are found to be inhibitors of protein kinase B/Akt (Zhu *et al*., 2007) and PDE4B (Mitchell *et al.,* 2010). It is well documented that incorporation of azo and hydrazono group has been reported to enhance the biological activity of heterocyclic compounds (Raman and Ravindernath, 1999). Compounds containing azo- and hydrazo-groups have been found to display many diverse types of biological properties such as antimicrobial (Shah *et al*., 2011), antifungal (Xu and Zeng, 2010), anticonvulsant (Kucukguzel *et al*., 2000), antioxidant and antitumor activity (Farghaly and Abdalla, 2009). Arylazopyrazoles are found to possess antioxidant as well as antibacterial activity (ManojKumar *et al*., 2009). Recently, a series of 4-arylazo-3,5-diamino-1*H*-pyrazoles derivatives have been reported as novel groups of ATP antagonists with moderate potency against CDK2-Cyclin E (Krystof *et al*., 2006). In view of these observations and in continuation of our work related to the synthesis, spectral and biological studies of aminopyrazoles (Aggarwal *et al*., 2006; Kumar *et al*., 2005), it was envisaged to undertake the synthesis and

R. Aggarwal (✉) · V. Kumar
Department of Chemistry, Kurukshetra University, Kurukshetra 136119, India
e-mail: ranjana67in@yahoo.com; ranjanaaggarwal67@gmail.com

G. K. Gupta
Department of Pharmacy, Maharishi Markandeshwar University, Mullana 133207, India

V. Kumar
Department of Chemistry, Maharishi Markandeshwar University, Mullana 133207, India

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antimicrobial activity study of 3,5-diamino-4-(4′-fluorophenylazo)-1-aryl/heteroarylpyrazoles.

Chemistry

Synthesis of 3,5-diamino-4-(4′-fluorophenylazo)-1-aryl/heteroarylpyrazoles (summarized in Scheme 1) was accomplished by a two step reaction. Initially, the key intermediate, 2-[(4-fluorophenyl)hydrazono]malononitrile **3** was prepared by the diazotization of 4-fluoroaniline **1**, followed by in situ condensation with malononitrile **2** in the presence of sodium acetate. Then, various aryl and heteroarylhydrazines **4a–4i** were reacted with this intermediate **3** in refluxing ethanol to yield the title compounds **5a–5i** as an exclusive product. The yields ranged from 68 to 83 %. The physical properties of the synthesized compounds are presented in a Table 1. The structure of **5a–5i** was established on the basis of analysis of IR, ^{1}H and ^{13}C NMR data.

The IR spectra of **5a–5i** revealed four characteristic absorption bands between 3,186 and 3,556 cm^{-1} due two NH_2 groups and a strong absorption band at about 1,490 cm^{-1} due to N=N stretch of the azo group and disappearance of absorption bands at 2,222 cm^{-1} due to C≡N stretch of **3**. The ^{1}H NMR spectra of **5a–5i** displayed the two multiplets each of two protons intensity at δ 7.29–7.34 and at δ 7.74–7.85 for 4-fluorophenylazo group as well as two broad singlets of two NH_2 groups (exchangeable with D_2O) in the range of δ 5.22–8.24 ppm. A singlet of three protons intensity at δ 2.42 and 2.74 ppm appeared due to $-CH_3$ group of **5f** and **5i**, respectively. Compound **5h** exhibited two sharp singlets: a singlet of six protons intensity at δ 2.45 for two methyl groups and one proton intensity due to pyrimidine 5-H at δ 6.8 ppm. Further support to the structure **5a–5i** was provided by ^{13}C NMR spectra. The ^{13}C NMR spectra of compounds **5a–5i** displayed signal for C_3, C_4, and C_5 at about δ 150–153, 114 and 148–151 ppm, respectively. It is well documented in literature that incorporation of amino group at position-3 and 5 bring a downfield shift of about δ 16–17 and 20–24 ppm for C_3 and C_5, respectively, and an upfield shift of about 13–17 ppm for C-4 carbon (Frigola *et al.*, 1989) and the present values are in complete agreement with earlier report. The complete assignment of the signals in ^{13}C NMR spectra of these compounds is given in Table 2.

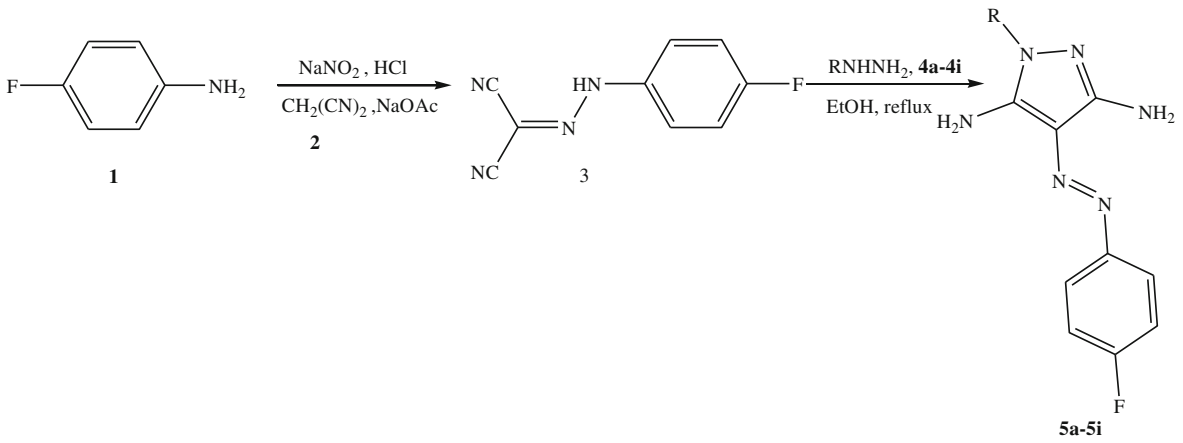
A plausible mechanism for the formation of **3** from malononitrile and diazonium salt of 4-fluoroaniline is outlined in Scheme 2.

Nucleophilic attack of sodium salt of malononitrile **6** (in situ generated in the separate flask by treatment of malononitrile **2** with sodium acetate), on diazonium salt **7** afforded **3**, which further cyclocondensation with binucleophilic hydrazine **4** to yield **5**.

Biological results and discussion

In vitro antibacterial activity

All the synthesized compounds **5a–5i** were screened in vitro for their antibacterial activity against two Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and two Gram-negative bacteria (*Escherichia coli*, *Klebesiella aerogenes*) (Tables 3, 4). The antibacterial activity of these compounds was compared with Cefixime as a standard drug. In general, the results revealed that most of the compounds showed better inhibition against Gram-negative rather than Gram-positive bacteria. All the compounds **5a–5i** showed minimum inhibitory concentration (MIC) ranging 2–32 μg/ml against Gram-negative bacterial strain *K. aerogenes*. The



Scheme 1 Synthesis of 3,5-diamino-4-(4′-fluorophenylazo)-1-aryl/heteroarylpyrazoles

Table 1 General structure and physical data of the synthesized compounds **5a–5i**

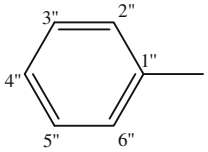
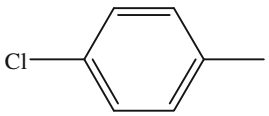
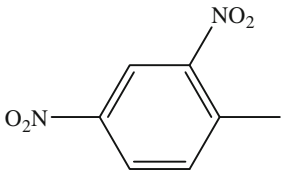
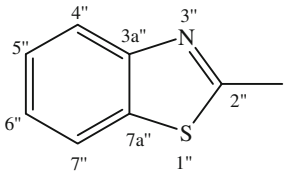
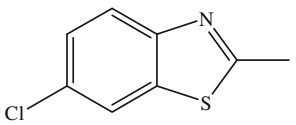
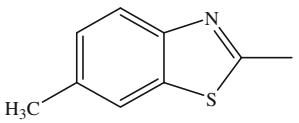
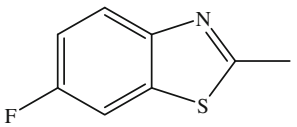
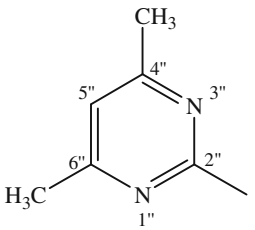
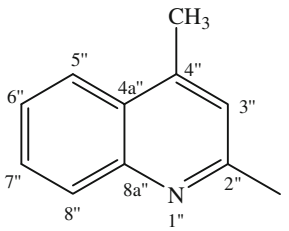
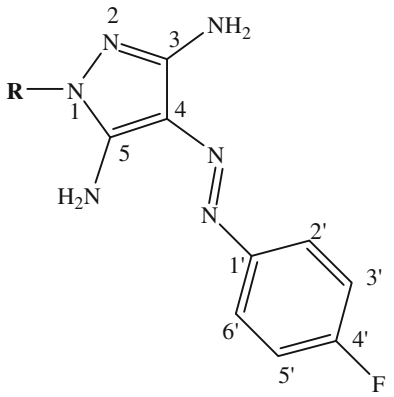
Compounds	R	Mol. formula (m.wt)	Elemental analysis (*N*) (%)		M.pt (°C)	Yield (%)
			Found	Calcd.		
5a		$C_{15}H_{13}FN_6$ (296.3)	28.41	28.36	184	70
5b		$C_{15}H_{12}ClFN_6$ (330.15)	25.47	25.41	180	73
5c		$C_{15}H_{11}FN_8O_4$ (386.3)	29.09	29.01	198	68
5d		$C_{16}H_{12}FN_7S$ (353.58)	28.03	27.75	262	83
5e		$C_{16}H_{11}ClFN_7S$ (387.82)	25.37	25.28	282	81
5f		$C_{17}H_{14}FN_7S$ (367.4)	27.05	26.69	278	75
5g		$C_{16}H_{11}F_2N_7S$ (371.37)	26.79	26.40	274	78
5h		$C_{15}H_{15}FN_8$ (326.33)	34.39	34.34	298	72
5i		$C_{19}H_{16}FN_7$ (361.38)	27.18	27.13	238	74

Table 2 ^{13}C NMR data for compounds **5a–5i** (ppm)



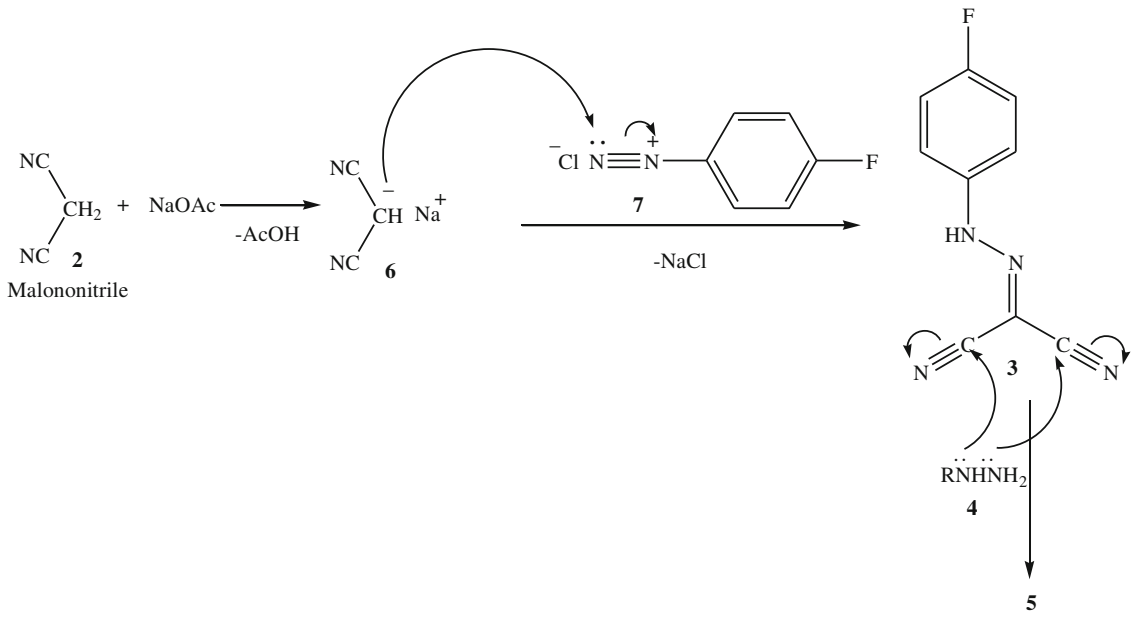
Carbon-atoms	5a	5b	5c	5d	5e	5f	5g	5h	5i
C-3	152.54	153.66	152.56	151.33	151.42	150.32	150.87	152.82	153.06
C-4	114.80	115.01	115.21	114.17	114.08	114.15	114.08	114.48	114.62
C-5	149.92	150.53	148.16	150.28	150.10	149.03	148.06	150.48	150.50
C-1′	122.42	122.89	122.47	123.35	122.25	121.10	122.12	122.80	122.93
C-2′, 6′	115.84	115.87	115.32	116.28	115.95	116.28	116.03	116.23	116.76
C-3′, 5′	115.54	116.49	115.78	115.98	115.63	115.98	115.74	115.93	115.92
C-4′	164.14	163.41	163.14	163.84	163.97	163.81	163.84	163.76	163.52
C-1″	160.85	160.17	161.88	–	–	–	–	–	–
C-2″	129.74	124.49	142.12	161.48	161.22	160.66	161.22	168.42	160.28
C-3″	122.87	129.60	119.34	–	–	–	–	–	112.65
C-4″	127.19	137.83	140.34	123.24	123.02	123.33	123.22	124.18	145.66
C-5″	122.87	129.60	127.84	127.06	127.07	128.30	127.98	123.00	123.04
C-6″	129.74	124.59	129.26	124.43	128.74	134.02	160.12	124.29	124.45
C-7″	–	–	–	121.48	121.58	122.09	122.42	–	128.19
C-8″	–	–	–	–	–	–	–	–	130.66
C-3a″	–	–	–	160.59	160.74	160.56	160.45	–	–
C-4a″	–	–	–	–	–	–	–	–	125.64
C-7a″	–	–	–	131.33	132.86	131.39	132.14	–	–
C-8a″	–	–	–	–	–	–	–	–	148.02
CH_3	–	–	–	–	–	21.43	–	23.96	19.22

C-1″ to C-8a″ are the carbon of R group

antibacterial activity of the tested compounds **5a**, **5c,** and **5i** against *E. coli* and **5b–5f** against *K. aerogenes* were comparable to the reference antibacterial drug Cefixime at MIC 2 μg/ml. The antibacterial activity of the tested compounds **5c**, **5g**, and **5i** against *B. subtilis* were similar to reference antibacterial drug Cefixime at MIC 4 μg/ml. Compound **5i** showed good broad spectrum activity against all the tested strains. Except for compounds **5h** and **5i,** all tested compounds were found to be inactive against *S. aureus* and it is interesting to note that **5i** displayed excellent activity as that of reference drug (Cefixime), however, **5h** showed moderate antibacterial activity.

In vitro antifungal activity

All the nine compounds **5a–5i** were tested for their antifungal activity in vitro against pathogenic fungi: *Aspergillus niger*, *Candida albicans* and *Saccharomyces cerevisiae* (Tables 3, 4) and Fluconazole was used as the standard antifungal drug. Compounds, **5c** and **5g** showed promising inhibition against the *A. niger* at MIC 2 μg/ml. Except **5g** and **5i**, almost all of the compounds showed potent inhibition against *C. albicans* with MIC range of 2 μg/ml which is comparable to reference antifungal drug (Fluconazole). Compound **5a** showed antifungal activity



Scheme 2 Mechanism of formation 3,5-diamino-4-(4′-fluorophenylazo)-1-aryl/heteroarylpyrazoles

Table 3 In vitro antibacterial and antifungal activities of synthesized compounds through agar well-diffusion method

Compounds	Diameter of growth of inhibition zone (mm)[a]						
	Antibacterial				Antifungal		
	S. aureus	*B. subtilis*	*K. aerogenes*	*E. coli*	*C. albicans*	*A. niger*	*S. cerevisiae*
5a	–	10	12	12	07	–	12
5b	–	–	12	–	09	–	–
5c	–	10	13	13	11	10	12
5d	–	09	14	12	12	–	09
5e	–	–	10	–	12	–	–
5f	–	–	10.5	–	11	–	–
5g	–	12	11	–	–	14	–
5h	4.5	–	09	–	09	–	10
5i	2.6	12	15	15	10	–	–
Cefixime	2.9	2.8	15	14	–	–	–
Fluconazole	–	–	–	–	10	13	12

–, No activity; *S. aureus*, *Staphylococcus aureus*; *B. subtilis*, *Bacillus subtilis*; *K. aerogenes*, *Klebsiella aerogenes*; *E. coli*, *Escherichia coli*; *C. albicans*, *Candida albicans*; *A. niger*, *Aspergillus niger*; *S. cerevisiae*, *Saccharomyces cerevisiae*

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

against *S. cerevisiae* comparable to the reference antifungal drug Fluconazole at MIC 4 μg/ml. Therefore, we concluded that almost all of the compounds found to possess good antifungal activity.

Structure–activity relationship

A careful analysis of the MIC data shows that replacement of the hydrogen in aryl group of compound **5a** by chlorine at postion-4 (**5b**) increases the antibacterial activity against *K. aerogenes* comparable to the reference drug (Cefixime). Also, incorporation of nitro group at postion-2 and -4 (**5c**) also shows a significant level of increase in antibacterial activity against *K. aerogens* and *B. subtilis* and antifungal activity against *A. niger* and *S. cerevisiae*. It is observed that replacement of aryl and benzothiazole ring at position-1 of pyrazole by 4″,6″-dimethylpyrimidin-2″-yl (**5h**) and by 4″-methylquinolin-2″-yl (**5i**) enhances the antibacterial activity against *S. aureus* (**5a–5g** vs **5h–5i**). Moreover, substitution of fluorine at position-6 of benzothiazole nucleus also results in increase in antifungal activity against *A. niger* and complete loss in activity against *C. albicans* amongst the benzothiazole series (**5g** vs **5d–f**).

The data reported in Tables 3 and 4 and Fig. 1 revealed that **5c** is the most active compound as compared to the other compounds. However, all these compounds represent promising new leads for combating the emerging pathogens.

Table 4 MIC of **5a–5i** against test bacteria and fungi using agar well-diffusion method

Compounds	MIC (μg/ml)[a]						
	S. aureus	*B. subtilis*	*K. aerogenes*	*E. coli*	*C. albicans*	*A. niger*	*S. cerevisiae*
5a	–	8	32	2	2	–	4
5b	–	–	2	–	2	–	–
5c	–	4	2	2	2	2	8
5d	–	6	2	8	2	–	6
5e	–	–	2	–	2	–	–
5f	–	–	2	–	2	–	–
5g	–	4	4	–	–	2	–
5h	16	–	8	–	2	–	8
5i	2	4	8	2	64	–	–
Cefixime	2	4	2	2	–	–	–
Fluconazole	–	–	–	–	2	2	4

[a] Mean of three replicates

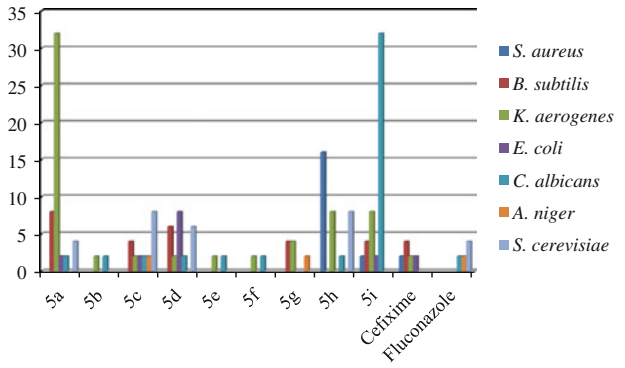


Fig. 1 Comparison of MIC of tested compounds with commercial antibiotics range MIC 2–32 (μg/ml)

Experimental

Melting points were determined in open capillaries with an electrical apparatus and are uncorrected. The IR spectra of the compounds were recorded on Buck Scientific IR M-500 spectrophotometer using KBr pellets (ν_{max} in cm^{-1}). ^1H and ^{13}C NMR spectra on a Bruker instrument at 300 and 75 MHz, respectively.

Arylhydrazines **4a–4c** were commercially available. Heteroarylhydrazines **4d–4i** (Potts *et al*., 1972; Katz, 1951; Kosolapoff and Roy, 1961) and 2-[(4-fluorophenyl) hydrazono]malononitrile **3** (Krystof *et al*., 2006) were prepared according to the literature procedure.

General procedure for the preparation of 3,5-diamino-4-(4′-fluorophenylazo)-1-aryl/heteroarylpyrazoles (**5a–5i**)

2-[(4-Fluorophenyl)hydrazono]malononitrile **3** (0.94 g, 5 mmol) was dissolved in 40 ml ethanol and an equivalent amount of appropriate hydrazines **4a–4i** (5 mmol) and 3–4 drops of acetic acid was added to it. The reaction mixture was refluxed for 2 h. The crude product so obtained on cooling was filtered and recrystallized from ethanol to give pure **5a–5i**.

Analytical and spectral data of synthesized compounds (**5a–5i**)

*3,5-Diamino-4-(4′-fluorophenylazo)-1-phenylpyrazole (**5a**)*

IR (KBr): 1,497 (N=N), 3,186, 3,294, 3,387, 3,464 (NH_2) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 5.22 (bs, 2H, exchangeable with D_2O), 6.98–7.05 (m, 2H), 7.19–7.49 (m, 5H), 7.54–7.60 (m, 2H). MS (EI): $[M+1]^+$, *m/z*, 297.5.

*3,5-Diamino-1-(4″-chlorophenyl)-4-(4′-fluorophenylazo)pyrazole (**5b**)*

IR (KBr): 1,489 (N=N), 3,256, 3,317, 3,425, 3,479 (NH_2) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 6.90 (bs, 2H, exchangeable with D_2O), 7.21–7.27 (m, 2H), 7.51–7.60 (m, 4H), 7.83–7.85 (m, 2H). MS (EI): $[M+1]^+$ and $[M+3]^+$, *m/z*, 331.2 and 333.2 in the ratio of 3:1 showing the typical isotopic profile.

*3,5-Diamino-1-(2″,4″-dinitrophenyl)-4-(4′-fluorophenylazo)pyrazole (**5c**)*

IR (KBr): 1,492 (N=N), 3,248, 3,344, 3,456, 3,464 (NH_2) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 5.03 (bs, 2H, exchangeable with D_2O), 7.65–7.68 (m, 2H), 8.23–8.26 (m, 2H), 8.80–8.86 (m, 2H), 9.99 (s, 1H). MS (EI): $[M+1]^+$, *m/z*, 386.3.

*3,5-Diamino-1-(benzothiazol-2″-yl)-4-(4′-fluorophenylazo)pyrazole (**5d**)*

IR (KBr): 1,489 (N=N), 3,256, 3,310, 3,379, 3,418 (NH_2) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 6.49 (bs, 2H, exchangeable

with D_2O), 7.26–7.37 (m, 3H), 7.46–7.51 (m, 1H), 7.85–7.88 (m, 3H), 8.01–8.03 (d, $J = 7.5$ Hz, 1H), 8.24 (bs, 2H, exchangeable with D_2O). MS (EI): $[M+1]^+$, *m/z*, 354.1.

*3,5-Diamino-1-(6″-chlorobenzothiazol-2″-yl)-4-(4′-fluorophenylazo)pyrazole (**5e**)*

IR (KBr): 1,497 (N=N), 3,232, 3,410, 3,472, 3,556 (NH_2) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 7.11–7.16 (m, 2H), 7.40–7.42 (d, $J = 7.5$ Hz, 1H), 7.74–7.78 (m, 4H), 7.88 (bs, 2H, exchangeable with D_2O), 8.04 (bs, exchangeable with D_2O). MS (EI): $[M+1]^+$ and $[M+3]^+$, *m/z*, 388.1 and 390 in the ratio of 3:1 showing the typical isotopic profile.

*3,5-Diamino-4-(4′-fluorophenylazo)-1-(6″-methylbenzothiazol-2″-yl)pyrazole (**5f**)*

IR (KBr): 1,497 (N=N), 3,256, 3,317, 3,379, 3,418 (NH_2) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.42 (s, 3H, CH_3), 6.47 (bs, 2H, exchangeable with D_2O), 7.26–7.32 (m, 3H), 7.73–7.76 (d, $J = 8.1$ Hz, 1H), 7.82 (s, 1H), 7.86–7.90 (m, 2H), 8.20 (bs, 2H, exchangeable with D_2O). MS (EI): $[M+1]^+$, *m/z*, 368.3.

*3,5-Diamino-1-(6″-fluorobenzothiazol-2″-yl)-4-(4′-fluorophenylazo)pyrazole (**5g**)*

IR (KBr): 1,489 (N=N), 3,256, 3,310, 3,371, 3,410 (NH_2) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 6.50 (bs, 2H, exchangeable with D_2O), 7.29–7.34 (m, 3H), 7.88–7.96 (m, 4H), 8.19 (bs, 2H, exchangeable with D_2O). MS (EI): $[M+1]^+$, *m/z*, 372.5.

*3,5-Diamino-4-(4′-fluorophenylazo)-1-(4″,6″-dimethylpyrimidin-2″-yl)pyrazole (**5h**)*

IR (KBr): 1,489 (N=N), 3,225, 3,302, 3,391, 3,418 (NH_2) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 2.47 (s, 6H, CH_3), 5.22 (s, 1H, exchangeable with D_2O), 6.73 (s, 1H), 6.99–7.05 (m, 2H), 7.18 (s, 1H, exchangeable with D_2O), 7.56–7.60 (m, 2H). MS (EI): $[M+1]^+$, *m/z*, 327.2.

*3,5-Diamino-4-(4′-fluorophenylazo)-1-(4″-methylquinolin-2″-yl)pyrazole (**5i**)*

IR (KBr): 1,497 (N=N), 3,186, 3,343, 3,418, 3,476 (NH_2) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.74 (s, 3H, CH_3), 7.25–7.31 (m, 2H), 7.53–7.58 (m, 1H), 7.74–7.80 (m, 2H) 7.83–7.88 (m, 2H), 8.03–8.07 (m, 2H). MS (EI): $[M+1]^+$, *m/z*, 362.2.

In vitro biological assays

Test microorganisms

Pathogenic bacterial strain namely *S. aureus* (Gram-positive), *B. subtilis* (Gram-positive), *E. coli* (Gram-negative), and *K. aerogenes* (Gram-negative) and three fungi, *A. niger*, *C. albicans*, and *S. cerevisiae)* were isolated from the patients in Maharishi Markandeshwar Medical College, Maharishi Markandeshwar University, Mullana, Haryana and were used in the present study.

In vitro antibacterial activity

The in vitro antibacterial activity of nine synthesized compounds was evaluated by the agar well-diffusion method (Sadashiva *et al*., 2004). 25 ml of nutrient agar medium was poured into each petri plate and the agar plates were swabbed with 100-µl inocula of each tested bacterium and kept for 15 min for adsorption. Using sterile cork borer of 8-mm diameter, wells were bored into the seeded agar plates and these were loaded with a 50-µl volume. Solutions of the tested compounds and standards were prepared in DMSO at concentration of 2,000 µg/ml. From this stock solution, twofold dilutions of the compounds (2, 4,…64 µg/ml) were inoculated to the corresponding wells. All the plates were incubated at 37 °C for 24 h. In vitro antibacterial activity of each synthesized compound was evaluated by measuring the zone of growth inhibition and MIC against the tested organisms with zone reader (Hi Antibiotic zone scale). MIC was determined as the lowest concentration of the compound tested that was able to inhibit visible growth of bacteria or fungi. Dimethylsulphoxide (DMSO) was used as a negative control, whereas Cefixime was used as a reference drug. The experiments were performed in triplicates.

In vitro antifungal activity

The in vitro antifungal activity of titled compounds was evaluated by the agar well-diffusion method (Sadashiva *et al*., 2004). The moulds were grown on Potato dextrose agar (PDA) at 25 °C for 7 days and used as inocula. 15 ml of molten PDA (45 °C) was poisoned by the addition of 50-µl volume of each compound, poured into a sterile petri plate and allowed to solidify at room temperature. Solutions of the tested compounds and standards were prepared in DMSO at concentration of 2,000 µg/ml. From this stock solution, twofold dilutions of the compounds (2, 4,…64 µg/ml) were inoculated to the corresponding wells. The solidified poisoned agar plates were inoculated at the centre with fungal plugs (8 mm diameter), obtained from the actively growing colony and incubated at 25 °C for

7 days. DMSO was used as the negative control, whereas Fluconazole was used as the reference drug. The experiments were performed in triplicates.

Conclusion

This study comprises the synthesis of 3,5-diamino-4-(4′-fluorophenylazo)-1-aryl/heteroarylpyrazoles. The antibacterial activity study of the tested compounds **5a**, **5c**, and **5i** against *E. coli*, **5b–5f** against *K. aerogenes* and **5c**, **5g,** and **5i** against *B. subtilis* were similar to the reference antibacterial compound (Cefixime). Except **5i**, almost all the compounds found to possess excellent level of antifungal activity comparable to standard drug Fluconazole.

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