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Fused Pyrimidine Hybrids Facile Synthesis, Characterization and *In vitro* Antimicrobial Screenings

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ABSTRACT

In the present study, we herein report the synthesis, characterization and in vitro antimicrobial investigations of 3-methyl-4-(substitutedphenyl)-1,7-diphenyl-1,4-dihydropyrazolo[3,4-d][1,3,4]-thiadiazolo[3,2-a]pyrimidine derivatives by following renowned Biginelli reaction using microwave irradiations. Agar Diffusion Method was employed to carry out anti-microbial activities. The synthesized fused systems were found to be entirely anti-bacterial in nature and compounds 4a, 4g possessing nitro and chloro substituent respectively, at ortho position on the aromatic ring at C-4, behaved as the most promising antibacterial moieties. None of the compounds showed antifungal activity.

Keywords: Fused pyrimidine scaffold, Environmentally benign procedure, Antibacterial agents

INTRODUCTION

Over decades, pyrimidine system is engaged as an important pharmacor, interacting with the synthesis and functioning of nucleic acids [1]. Because of versatile chemical reactivity and wide spectrum of biological behaviours, pyrimidine derivatives have attracted medicinal chemists in the pharmaceutical field. Among five membered heterocycles, 1,3,4-thiadiazole nucleus is an integral part of various natural products and medicinally significant compounds. This ring system possesses diversified biological activities such as antidiabetic [2], antitubercular [3], anti-inflammatory [4], and diuretic agent [5]. Pyrazolone system has been found to be associated with multiple pharmacological activities such as anticonvulsant [6], anti-inflammatory [7], antimicrobial and many more.

Condensed pyrimidine framework has been found to exhibit various pharmaceutical applications. Pyrazolo[3,4-d]pyrimidine derivatives have emerged as potent antitumor agents against Human Breast Adenocarcinoma (MCF-7) cell line [8-10] and Hep-2 [11] and they have shown anti-proliferative and antioxidant behaviour [12] as well. Antimicrobial and antioxidant activity [13] has been found to be associated with thiazolopyrimidine, thiazolodipyrimidine and thiazolopyrimidopyrimidine derivatives. Antitumor [14] behaviour against MCF-7, Hep-G2 cell line has been found to be possessed by thiazoles, thiadiazoles, pyrimido[2,3-d][1,2,4]-triazolo[4,3-a]pyrimidine derivatives.

In previous year, we synthesised bridgehead thiadiazolo[3,2-a]pyrimidine derivatives [15] and pyrazolo[3,4-d]pyrimidine derivatives [16] and among those, formerly described moieties exhibited potent antibacterial activity against *B. subtilis* and *P. glabrum* and latterly mentioned moieties were found to possess promising anti-fungal behaviour.

Victorious pharmaceutical applications of fused pyrimidine scaffold prompted us to adopt a new series with a hope that the resulting hybrid heterocycle will exhibit enhanced biological significance in the field of medicinal chemistry. In continuation of our ongoing research work, we herein report the synthesis of 3-methyl-4-(substitutedphenyl)-1,7-diphenyl-1,4-dihydropyrazolo[3,4-d][1,3,4]-thiadiazolo[3,2-a]pyrimidine derivatives using 1,3,4-thiadiazole ring and pyrazolone as significant precursors. Microwave Assisted Organic Synthesis (MAOS) has been adopted as green and environmentally friendly procedure to fulfill this purpose.

MATERIALS AND METHODS

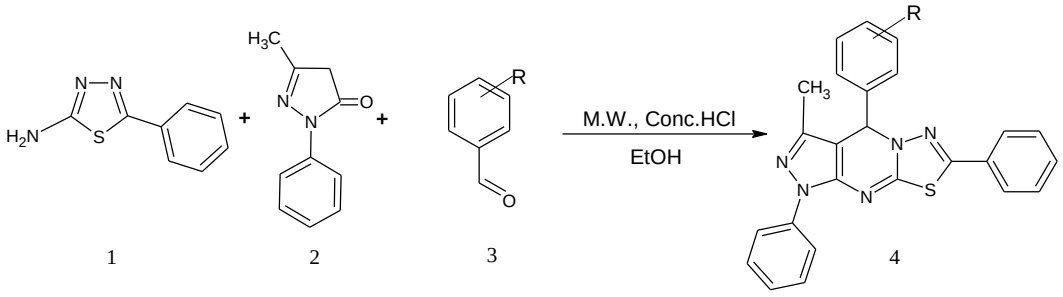
Instrumentation and techniques employed

Melting points were determined on Griffin-Kamp M.P. apparatus and are uncorrected. All synthesized compounds were characterised by IR, ^{1}H-NMR, ^{13}C-NMR, elemental analyses. IR spectra were recorded as potassium bromide pellets on Perkin Elmer spectrum RX FTIR System. ^{1}H-NMR and ^{13}C-NMR spectra were determined on Bruker Avance II 400 NMR Spectrometer. Chemical shifts were expressed as parts per million; (δ values, ppm) relative to Tetramethylsilane (TMS) as internal standard. The mass spectra were recorded by GC-MS-QP2010 Plus (Shimadzu Corporation, Kyoto, Japan). The elemental analyses were recorded on Vario Micro C, H, N, S Analyzer. The synthesized compounds gave satisfactory results within ± 0.4% of theoretical values. Analytical Thin Layer Chromatography (TLC) was employed to follow course of

reaction and to check the purity of products, on silica gel (60, GF254, Merck) using glass plates. The spots were visualized by exposure to iodine vapours. The reactions using microwaves had been carried out using scientific microwave oven, M.W. 300, Anton Paar. The biological evaluation of the synthesised compounds was conducted at Biogenic Research and Training Centre in Biotechnology, Hubli, Karnatka.

EXPERIMENTAL SECTION

Synthetic scheme followed



1 2 3 4

R = H, 2-NO_2, 3-NO_2, 4-NO_2, 2,4-Cl, 4-OH, 3-OCH_3, 4-OH, 2,3-OCH_2O, 4-OCH_3, 2-Cl, 4-Cl, 3-OH

General method for the preparation of 2-amino-5-phenyl-1,3,4-thiadiazole (1) [17-19]

A mixture of benzoic acid (0.01 mol), thiosemicarbazide (0.01 mol) and phosphorus oxychloride (5 ml) was refluxed gently for 3 h. After cooling the reaction mixture, 25 ml of water was added and reaction mixture was again refluxed for another 3 h. Solid mass thus separated during reaction was then separated by filtration and the filtrate so obtained was neutralized using concentrated potassium hydroxide solution. On neutralization, yellow colored precipitates were obtained which were then filtered, dried and recrystallized using equimolar mixture of methanol and chloroform. Crystalline solid was obtained with m.p. 220-222°C and yield was 80%.

General method for the preparation of 3-methyl-1-phenyl-pyrazol-5-one (2) [20]

An equimolar mixture of pure ethylacetoacetate (0.05 mol) and phenyl hydrazine (0.05 mol), containing few drops of glacial acetic acid as catalyst, was irradiated using microwave irradiations in the presence of absolute alcohol as energy transfer medium, for appropriate time. The progress of reaction was followed by TLC. The solid thus separated upon cooling the reaction mixture at room temperature, was filtered, dried and recrystallized using ethanol. Creamish white solid was obtained with m.p. 126°C and yield was 85%.

General method for the preparation of 3-methyl-4-(substitutedphenyl)-1,7-diphenyl-1,4-dihydropyrazolo[3,4-d][1,3,4]-thiadiazolo[3,2-a]pyrimidine (4)

An equimolar mixture of 1 (1.77 g, 0.01 mol), 2 (1.75 g, 0.01 mol) and an appropriate aromatic aldehyde 3 (0.01 mol), using absolute alcohol (15 ml) as energy transfer medium in the presence of a few drops of conc. HCl, was irradiated using microwaves. The reaction mixture was then kept undisturbed overnight after ensuring the completion of reaction using TLC. The obtained precipitates were filtered, dried and recrystallized using appropriate solvent. The physical characterization data has been compiled up and represented in Table 1.

Table 1: Physical characterization data of compounds 4(a-l)

S. No.	Compound	R	Time (MW irradiations)	Colour	Melting point (°C)	Molecular formula	Yield (%)
1	4a	2-NO_2	25 min	yellow	140-142	$C_{25}H_{18}N_6SO_2$	60-63
2	4b	3 -NO_2	15 min	yellow	142-144	$C_{25}H_{18}N_6SO_2$	65-67
3	4c	4-NO_2	15 min	orange	208-210	$C_{25}H_{18}N_6SO_2$	60-62
4	4d	4-OH	32 min	orange	212-214	$C_{25}H_{19}N_5SO$	55-57
5	4e	3-OH	33 min	yellow	80-82	$C_{25}H_{19}N_5SO$	60-62
6	4f	4-Cl	10 min	yellow	240-242	$C_{25}H_{18}N_5SCl$	53-55
7	4g	2-Cl	12 min	yellow	212-214	$C_{25}H_{18}N_5SCl$	53-55
8	4h	2,4-Cl	15 min	yellow	144-146	$C_{25}H_{17}N_5SCl_2$	50-52
9	4i	H	35 min	orange	174-176	$C_{25}H_{19}N_5S$	60-63
10	4j	4-OH,3-OCH_3	15 min	orange	168 -170	$C_{26}H_{19}N_5SO_2$	68-70
11	4k	4-OCH_3	26 min	yellow	130-132	$C_{26}H_{18}N_5SO$	62-65
12	4l	2,3-OCH_2O	25 min	orange	158-160	$C_{26}H_{19}N_5SO_2$	60-63

Spectral analysis

3-methyl-4-(2-nitrophenyl)-1,7-diphenyl-1,4-dihydropyrazolo[3,4-d][1,3,4]-thiadiazolo[3,2-a]pyrimidine (4a): Orange solid. Yield: 60-63%; m.p. 140-142°C. IR (KBr, cm^{-1}): 3072, 2910, 2885, 1687, 1600, 1527, 1455, 1348; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ=7.80-6.82 (m, 14H), 4.9 (s, 1H), 2.3 (s, 3H). ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ=152.7, 148.0, 145.7, 141.0, 138.9, 131.5, 129.2 (2C), 127.9 (2C), 127.5 (2C), 126.5 (2C), 123.9 (2C), 123.5 (2C), 118.5, 61.0, 13.0. GC-MS; Mass fragments (m/z): 489 (M+Na), 291 (100%), 234, 221, 186, 159, 158, 145, 131, 130, 104, 103, 91, 77, 51. Anal. calc. for $C_{25}H_{18}N_6SO_2$: C, 64.36%; H, 3.89%; N, 18.01%; O, 6.86%; S, 6.87%. Found: C, 63.96%; H, 4.29%; N, 18.41%; O, 6.46%; S, 6.47%.

3-methyl-4-(3-nitrophenyl)-1,7-diphenyl-1,4-dihydropyrazolo[3,4-d][1,3,4]-thiadiazolo[3,2-a]pyrimidine (4b): Yellow solid. Yield: 65-67%; m.p. 142-144°C. IR (KBr, cm^{-1}): 3068, 2920, 2743, 1697, 1523, 1450, 1346; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ=8.01-7.07 (m, 14H), 4.81 (s, 1H), 2.13 (s, 3H). ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ=157.3, 148.0, 104.5, 58.27, 18.29. GC-MS; Mass fragments (m/z): 489 (M+Na), 466 (M^+), 355, 307, 260, 232, 185 (100%), 174, 128, 91, 77, 51. Anal. calc. for $C_{25}H_{18}N_6SO_2$: C, 64.36%; H, 3.89%; N, 18.01%; O,

6.86%; S, 6.87%. Found: C, 64.76%; H, 4.29%; N, 17.61%; O, 7.26%; S, 6.47%.

3-methyl-4-(4-nitrophenyl)-1,7-diphenyl-1,4-dihydropyrazolo[3,4-d][1,3,4]-thiadiazolo[3,2-a]pyrimidine (4c): Yellow solid. Yield: 60-62%; m.p. 208-210°C. IR (KBr, cm^{-1}): 3050, 2915, 2880, 1545, 1690, 1601, 1470, 1345; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ=8.19 (dd, $J_{o,m}$=6.8 Hz, 1.8 Hz, 2H), 7.82 (dd, $J_{o,m}$=6.8 Hz, 1.8 Hz, 2H), 7.6-6.8 (m, 10H), 5.13 (s, 1H), 2.3 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ=153.8, 148.2, 145.9, 142.1, 139.7, 130.6, 129.3 (2C), 127.8 (2C), 127.6, 126.2, 123.7 (2C), 119.0, 61.9, 12.3. GC-MS; Mass fragments (m/z): 468 (M+2), 420, 405, 231 (100%), 154, 104, 91, 77, 51. Anal. calc. for $C_{25}H_{18}N_6SO_2$: C, 64.36%; H, 3.89%; N, 18.01%; O, 6.86%; S, 6.87%. Found: C, 64.76%; H, 3.49%; N, 17.61%; O, 7.26%; S, 7.27%.

3-methyl-4-(4-hydroxyphenyl)-1,7-diphenyl-1,4-dihydropyrazolo[3,4-d][1,3,4]-thiadiazolo[3,2-a]pyrimidine (4d); Orange solid, Yield: 55-57%; m.p. 212-214°C. IR (KBr, cm^{-1}): 3350, 2925, 2750, 1685, 1446, 1145; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ=9.21 (brs, 1H, OH, Exchanged by D_2O), 8.15-7.25 (m, 15H), 4.9 (s, 1H), 2.4 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ=156.5, 154.8, 148.0, 142.8, 142.7, 142.0, 139.7, 131.2, 131.0, 129.4 (2C), 129.3 (2C), 128.4 (2C), 127.9 (2C), 126.1, 124.0 (2C), 118.9, 115.8, 61.9, 12.9. GC-MS; Mass fragments (m/z): 437 ($M^{\cdot+}$), 460 (M+Na), 355, 249, 207, 185, 145, 115, 91, 77, 51. Anal. calc. for $C_{25}H_{19}N_5SO$: C, 68.63%; H, 4.38%; N, 16.01%; O, 3.66%; S, 7.33%. Found: C, 68.23%; H, 3.98%; N, 16.41%; O, 3.26%; S, 6.93%.

3-methyl-4-(3-hydroxyphenyl)-1,7-diphenyl-1,4-dihydropyrazolo[3,4-d][1,3,4]-thiadiazolo[3,2-a]pyrimidine (4e). Yellow solid, Yield: 60-62%; m.p. 80-82°C. IR (KBr, cm^{-1}): 3370, 3220, 2975, 2880, 1697, 1450, 1166; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ=9.8 (brs, 1H, OH, Exchanged by D_2O), 7.68-7.43 (m, 14H), 4.89 (s, 1H), 2.31 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ=156.8, 156.8, 147.1, 143.5, 142.1, 139.7, 131.0, 130.6, 129.9, 129.3 (2C), 129.2 (2C), 128.8 (2C), 126.2 (2C), 123.9 (2C), 119.5, 119.0, 113.9, 112.6, 62.2, 12.7. GC-MS; Mass fragments (m/z): 437 ($M^{\cdot+}$), 439 (M+2), 251, 188, 174, 91, 51. Anal. calc. for $C_{25}H_{19}N_5SO$: C, 68.63%; H, 4.38%; N, 16.01%; O, 3.66%; S, 7.33%. Found: C, 69.03%; H, 4.78%; N, 15.61%; O, 3.26%; S, 7.73%.

3-methyl-4-(4-chlorophenyl)-1,7-diphenyl-1,4-dihydropyrazolo[3,4-d][1,3,4]-thiadiazolo[3,2-a]pyrimidine (4f); Yellow solid, Yield: 53-55%; m.p. 240-242°C. IR (KBr, cm^{-1}): 3065, 2925, 2865, 1697, 1452, 1142; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ=8.32 (dd, $J_{o,m}$=6.8 Hz, 1.8 Hz, 2H), 7.96 (dd, $J_{o,m}$=6.8 Hz, 1.8 Hz, 2H), 7.5-6.2 (m, 10H), 5.12 (s, 1H), 2.3 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ=153.9, 146.8, 143.6, 142.5, 140.2, 139.8, 133.0, 132.3, 131.0, 130.8, 129.3 (2C), 129.2 (2C), 128.8 (2C), 128.6 (2C), 128.3 (2C), 126.2, 119.5, 61.9, 13.6. GC-MS; Mass fragments (m/z): 455 (M+), 457 (M+2), 323, 212, 172, 135, 74 (100%), 51. Anal. calc. for $C_{25}H_{18}ClN_5S$: C, 65.85%; H, 3.98%; N, 15.36%; Cl, 7.78%; S, 7.03%. Found: C, 65.45%; H, 4.38%; N, 14.96%; Cl, 8.18%; S, 7.43%.

3-methyl-4-(2-chlorophenyl)-1,7-diphenyl-1,4-dihydropyrazolo[3,4-d][1,3,4]thiadiazolo[3,2-a]pyrimidine (4g): Yellow solid, Yield: 53-55%; m.p. 212-214°C. IR (KBr, cm^{-1}): 3076, 2912, 2880, 1687, 1450, 1150; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ=7.84-7.08 (m, 14H), 4.8 (s, 1H), 2.4 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ=153.6, 143.6, 142.1, 139.5, 132.4, 131.0, 130.5, 129.2, 129.1, 128.8, 128.6, 128.1, 127.0, 126.6, 119.2, 56.5., 12.9. GC-MS; Mass fragments (m/z): 498 (M+Na), 455 ($M^{\cdot+}$), 457 (M+2), 439, 404, 264 (100%), 187. Anal. calc. for $C_{25}H_{18}ClN_5S$: C, 65.85%; H, 3.98%; N, 15.36%; Cl, 7.78%; S, 7.03%. Found: C, 66.25%; H, 3.58%; N, 15.76%; Cl, 7.38%; S, 6.63%.

3-methyl-4-(2,4-dichlorophenyl)-1,7-diphenyl-1,4-dihydropyrazolo[3,4-d][1,3,4]thiadiazolo[3,2-a]pyrimidine (4h). Yellow solid, Yield: 50-52%; m.p. 144-146°C. IR (KBr, cm^{-1}): 3054, 2927, 2860, 1682, 1447, 1166; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ=8.04-7.60 (m, 10H), 7.55 (d, J_m=2.8 Hz, 1H), 7.42 (dd, $J_{o,m}$=8.2 Hz, 2.0 Hz, 1H), 7.38 (d, J_o=8.4 Hz, 1H), 5.14 (s, 1H), 1.93 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ=152.9, 143.6, 142.0, 139.8 (2C), 133.7, 133.5, 130.1, 129.7, 129.4 (2C), 129.3 (2C), 128.6 (2C), 126.8, 126.4 (2C), 123.6 (2C), 118.7, 57.8, 12.8. GC-MS; Mass fragments (m/z): 490 ($M^{\cdot+}$), 492 (M+2), 475 (100%), 304, 227, 187, 91, 77, 51. Anal. calc. for $C_{25}H_{17}Cl_2N_5S$: C, 61.23%; H, 3.49%; N, 14.28%; Cl, 14.46%; S, 6.54%. Found: C, 61.63%; H, 3.89%; N, 13.88%; Cl, 14.86%; S, 6.14%.

3-methyl-1,4-diphenyl-1,4-dihydropyrazolo[3,4-d][1,3,4]thiadiazolo[3,2-a]pyrimidine (4i). Orange solid, Yield: 60-63%; m.p. 174-176°C. IR **(**KBr, cm^{-1}): 3054, 2927, 2860, 1682, 1447, 1166; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ=8.15-7.25 (m, 15H), 4.9 (s, 1H), 2.4 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ=153.7, 147.0, 143.6, 142.0, 131.0, 129.4 (2C), 129.3 (2C), 129.2 (2C), 129.0 (2C), 128.5 (2C), 126.9 (2C), 126.7 (2C), 126.7 (2C), 126.2 (2C), 119.2, 13.1. GC-MS; Mass fragments (m/z): 421, 444 (M+Na), 232, 209, 177, 135, 104, 74, 51. Anal. calc. for $C_{25}H_{19}N_5S$: C, 71.23%; H, 4.54%; N, 16.61%; S, 7.61%. Found: C, 70.83%; H, 4.14%; N, 17.01%; S, 7.21%.

3-methyl-4-(4-hydroxy,3-methoxy)-1,7-diphenyl-1,4-dihydropyrazolo[3,4-d][1,3,4]thiadiazolo[3,2-a]pyrimidine (4j): Orange solid, Yield: 68-70%; m.p. 168-170°C. IR (KBr, cm^{-1}): 3352, 2918, 2778, 1682, 1599, 1442, 1166, 1251, 1038; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ=8.8 (brs, 1H, OH, Exchanged by D_2O), 8.04-7.40 (m, 10H), 6.84 (d, J_m=1.1 Hz), 6.72 (d, J_o=8.0 Hz), 6.62 (d, J_o=6.8 Hz), 5.38 (brs, 1H), 5.19 (s, 1H), 3.83 (s, 3H), 2.3 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ=152.9, 147.3, 146.8, 146.5, 143.5, 141.9, 138.9, 135.7, 131.2, 129.5 (2C), 129.3 (2C), 128.5 (2C), 126.2 (2C), 123.9 (2C), 120.6 (2C), 115.4, 112.4, 62.2, 56.1, 13.1. GC-MS; Mass fragments (m/z): 481 (M^+), 483 (M+2), 306 (100%), 290, 229, 150, 77. Anal. calc. for $C_{26}H_{21}N_5SO_2$: C, 66.79%; H, 4.53%; N, 14.98%; O, 6.84%; S, 6.86%. Found: C, 66.39%; H, 4.13%; N, 15.38%; S, 6.46%; O, 6.44%.

3-methyl-4-(4-methoxyphenyl)-1,7-diphenyl-1,4-dihydropyrazolo[3,4-d][1,3,4]thiadiazolo[3,2-a]pyrimidine (4k): Yellow solid. Yield: 62-65%; m.p. 130-132°C. IR (KBr, cm^{-1}): 3072, 2927, 2885, 1690, 1597, 1450, 1300, 1250; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ=8.17-7.51 (m, 14H), 4.31 (brs, 3H), 3.62 (s, 3H), 2.54 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ=164.2, 160.1, 147.9, 141.2, 134.28, 132.6, 130.2, 129.3 (2C), 129.1 (2C), 128.4 (2C), 126.8 (2C), 126.8 (2C), 126.4 (2C), 123.9 (2C), 122.5 (2C), 102.3, 61.7, 51.5, 19.3. GC-MS; Mass fragments (m/z): 451 (M^+), 429, 355, 341, 281, 267, 207 (100%), 191, 177, 147, 133, 96, 73. Anal. calc. for $C_{26}H_{18}N_5SO$: C, 69.16%; H, 4.69%; N, 15.51%; O, 3.545%; S, 7.10%. Found: C, 69.56%; H, 4.29%; N, 15.11%; S, 6.70%; O, 3.94%.

4-(benzo[d][1,3]-dioxo-4-yl)-3-methyl-1-phenyl-1,4-dihydropyrazolo[3,4-d][1,3,4]thiadiazolo[3,2-a]pyrimidine 4(l): Orange solid, Yield: 60-63%; m.p. 158-160°C. IR (KBr, cm^{-1}): 3065, 2930, 2890, 1690, 1448, 1145; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ=8.15-7.69 (m, 13H), 6.07 (s, 2H), 4.9 (s, 1H), 2.4 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ=153.6, 149.7, 148.1, 142.9, 142.1, 142.0, 139.6, 130.5, 129.4 (2C), 129.3 (2C), 128.5 (2C), 123.8 (2C), 126.2, 121.8, 121.6, 119.0, 118.3, 113.3, 56.3, 12.9. GC-MS; Mass fragments (m/z): 465, 488 (M+Na), 355, 278, 185 (100%). Anal. calc. for $C_{26}H_{19}N_5SO_2$: C, 67.08%; H, 4.11%; N, 15.04%; O, 6.87%; S, 6.89%. Found: C, 67.48%; H, 4.51%; N, 15.51%; S, 6.49%; O, 6.47%.

Biological assay

Antimicrobial screenings

The novel synthesized fused pyrimidine derivatives (4a-4l) were screened for their anti-bacterial behaviour using Gram-negative bacterial strains namely, *Escherichia coli*, *Pseudomonas aeruginosa* and Gram-positive bacteria; *Bacillus subtilis* and *Staphylococcus aureus* and in the similar

manner, *Candida albicans* and *Aspergillus flavus* had been employed as fungal strains to carry out antifungal activity. Agar diffusion method, using Dimethyl Sulfoxide (DMSO) as solvent and nutrient agar medium, was performed in inhibition assay to determine the average diameter of inhibition zones (in mm) of bacterial and fungal growth. Ciprofloxacin and fluconazole had been used as standard drugs for screening of antibacterial and antifungal activities respectively.

Methodology employed

Initially, the stock cultures of bacteria and fungi were revived by inoculating in broth media and grown at 37°C and 27°C for 18 and 48 h respectively. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated, with old cultures (100 µl, 10^{-4} cfu) and spread evenly on the plate. After 20 min, the wells were filled with compound at different volumes. All the plates were incubated at 37°C for 24 h for bacterial and at 27°C for 96 h, for fungal strains respectively and the diameter of inhibition zone were noted in mm at various concentrations. The results of anti-microbial screenings have been compiled up and given in Tables 2 and 3.

Table 2: *In vitro* antimicrobial evaluations of compounds 4(a-l) (Zone of inhibition in mm, at 500, 1000 µg/ml)

Compound tested	Bacterial strains								Fungal strains			
	B1		B2		B3		B4		F1		F2	
	500	1000	500	1000	500	1000	500	1000	500	1000	500	1000
4a	3	5	0	0	0	7	0	4	0	0	0	0
4b	0	4	0	3	0	0	0	0	0	0	0	0
4c	0	0	0	5	0	6	6	3	0	0	0	0
4d	0	0	0	0	0	0	0	0	0	0	0	0
4e	0	0	0	3	0	0	0	0	0	0	0	0
4f	0	3	0	0	0	2	0	2	0	0	0	0
4g	0	4	0	6	0	6	0	5	0	0	0	0
4h	0	4	0	6	0	6	0	5	0	0	0	0
4i	0	0	0	0	0	3	0	0	0	0	0	0
4j	0	6	0	2	0	3	0	0	0	0	0	0
4k	0	0	0	3	0	0	0	0	0	0	0	0
4l	0	5	0	3	0	3	0	0	0	0	0	0
Ciprofloxacin	27	*	28	*	22	*	8	*	-	-	-	-
Fluconazole	-	-	-	-	-	-	-	-	13	5	15	7

(*) denotes zones could not be found because of merging; (-) denotes no activity; Bacterial strains used: B1 (*Staphylococcus aureus*), B2 (*Escherichia coli*), B3 (*Bacillus subtilis*), B4 (*Pseudomonas aeruginosa*); Fungal strains used: F1 (*Candida albicans*), F2 (*Aspergillus flavus*)

Table 3: *In vitro* antimicrobial evaluations of compounds 4(a-l), Minimum Inhibitory Concentration (MIC), µg/ml)

Compound tested	B1	B2	B3	B4	F1	F2
4a	1000	0	1000	1000	0	0
4b	1000	1000	0	0	0	0
4c	0	1000	1000	1000	0	0
4d	0	0	0	0	0	0
4e	0	1000	0	0	0	0
4f	1000	0	0	0	0	0
4g	1000	1000	1000	1000	0	0
4h	1000	1000	1000	1000	0	0
4i	0	0	1000	0	0	0
4j	1000	0	1000	0	0	0
4k	0	1000	0	1000	0	0
4l	1000	1000	1000	0	0	0
Ciprofloxacin	25	25	25	0	-	-
Fluconazole	-	-	-	-	28	28

Bacterial strains used: B1 (*Staphylococcus aureus*), B2 (*Escherichia coli*), B3 (*Bacillus subtilis*), B4 (*Pseudomonas aeruginosa*); Fungal strains used: F1 (*Candida albicans*), F2 (*Aspergillus flavus*); (-) denotes no activity; MIC was not determined for those derivatives, whose zone of inhibition was less than 3 mm

RESULTS AND DISCUSSION

The purpose of this research was to design, synthesize and evaluate the anti-microbial activities of the 3-methyl-4-(substitutedphenyl)-1,7-diphenyl-1,4-dihydropyrazolo[3,4-d][1,3,4]-thiadiazolo[3,2-a]pyrimidine derivatives. And these fused pyrimidines were synthesized using thiadiazole, pyrazole-5-one and appropriate aromatic aldehydes by well-known Biginelli reaction [21]. The equimolar quantities of these precursors were condensed together using microwave irradiations for appropriate time as an ecofriendly benign method, in presence of absolute alcohol as energy transfer medium and few drops of conc. HCl as catalyst. The structures of prepared pyrimidine derivatives were confirmed by spectral characterization (IR, ^{1}H-NMR, ^{13}C-NMR, GC-MS). IR spectra showed stretching frequency from 1697-1680 cm^{-1}, characteristic of C = N stretching. Among NMR spectra, presence of characteristic singlet from about 5.2-4.9 ppm, in PMR and presence of resonance ranging from 62.2-56.1 ppm, among ^{13}C-NMR confirms the formation of pyrimidine ring. Rest of the characteristic absorption bands among IR and resonances in NMR spectra were in good agreement with proposed structures.

Antimicrobial investigations

Synthesized compounds were screened for their anti-microbial activities. Antibacterial and anti-fungal behaviour were evaluated by agar diffusion method in which MIC and zone of inhibition had been determined. It was observed that nature and position of the substituent attached to aromatic ring had promising influence on the biological behaviours of the synthesized derivatives. Order of effect of various substituents attached to the aromatic ring, was found to be as: NO_2 > Cl > 4-OH, 3-OCH_3 > 2, 3-OCH_2O > 4-OCH_3 > 3-OH > H. So, from the results of anti-microbial screening, we observed that pyrimidine moieties possessing electron-withdrawing group were more potent anti-bacterial agents than those which carry electron-releasing substituent. And among electron-withdrawing groups, that carrying nitro substituent, were the most

efficient. Further, position of the attached substituent, too, had great impact on the biological activities. Among the nitro groups, the prepared derivative having nitro group at ortho position was the most efficient antibacterial agent than those having this group at meta and para position. Similarly, among the chloro-substituted derivatives, moiety having chloro substituent at ortho position behaved very well. Therefore, it was found on the basis of above discussion that compound 4a was found to be the most potent anti-bacterial agent, followed by 4g and they exhibited 7 mm and 6 mm zone of inhibition against *B. subtilis* at 1000 µg/ml. And the compound 4i carrying no substituent on aromatic ring attached to C-4, behaved as weak antibacterial agent with zone of inhibition 3 mm at 1000 µg/ml against *B. subtilis*. However, none of the compounds was found to be antifungal in nature.

CONCLUSION

3-methyl-4-(substitutedphenyl)-1,7-diphenyl-1,4-dihydropyrazolo[3,4-d][1,3,4]-thiadiazolo[3,2-a]pyrimidine derivatives were synthesized and evaluated for their antimicrobial activities. From the results of antimicrobial evaluations, it was concluded that nature and position of substituents attached to aromatic ring had marked effect on the biological behaviours. Compound 4a having nitro substituent at ortho position was found to be the most promising antibacterial agent against *B. subtilis*. However, none of the synthesized compound had shown antifungal activity.

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