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Research Article

Synthesis of Novel 1,2,4-Triazole Derivatives as Antimicrobial Agents via the Japp-Klingemann Reaction: Investigation of Antimicrobial Activities

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In the present investigation, 1,2,4-triazole appended to pyrazoline and pyrazole rings (**4a–g**) using N-arylsydnone as synthon was prepared. The title compounds were subjected to Osiris property explorer for the oral bioavailability to analyze their drug likeness and drug score. Further, the compounds were subjected to the antimicrobial activity and analyzed the IC 50 and MIC values.

1. Introduction

3-Arylsydnone, a class of mesoionic compounds, has been used as synthon extensively for the synthesis of various pharmaceutically potent molecules like pyrazole, phenylindazole, carbazole, pyrazoline, tetrazine, and 1,3,4-oxadiazole by 1,3-dipolar cycloaddition and addition elimination reactions [1–3]. On ring insertion with hydrazine hydrate, 1,3,4-oxadiazole yields 1,2,4-triazole derivative [4]. 1,2,4-Triazolinone derivatives such as azafenidin and sulfentrazone have been reported as herbicides [5–9]. Synthesis of 1,2,4-triazoles fused to another heterocyclic ring has attracted wide spread attention due to their diverse applications as antibacterial, antidepressant, antiviral, antitumor, anti-inflammatory agents, pesticides, herbicides, dyes, lubricant, and analytical reagents [10].

There is a considerable interest in chemotherapeutic activity of pyrazole derivatives. They have been reported to exhibit broad spectrum of biological effects [11]. Pyrazoles also possess a broad spectrum of biological effectiveness such as antidepressant [12] and antibacterial activity [13]. Besides, great interest in the pyrazole molecule has been stimulated by some promising agrochemical applications such as herbicides and fungicides [14–16]. Pyrazole-1-carboxamidopyrazole and 1-thiocarbamoyl-pyrazole showed impressive *in vivo* antitumour activity in experimental animals. One of the series of azoxypyrazoles, that is, 1,1′-bis(2-hydroxyethyl)-3,5,3′,5′-tetra methylazoxypyrazole, has been reported to possess activity against ascitic forms of the *Ehrlich, Landschiitz, and Sarcoma 180* tumours and against the P.388 lymphatic leukaemia in mice [17]. In view of these observations, we herein report the synthesis of novel molecular scaffolds containing pyrazole, pyrazolines, and 1,2,4-triazole ring using N-arylsydnone as a synthon in the hope to get lead compounds as antimicrobial agents.

2. Experimental

Melting points were determined in open capillaries. The IR spectra were recorded on Nicolet Impact 5200 USA FT IR using KBr pellets. ^{1}H NMR spectra were recorded on Bruker Varian 300-MHz FT NMR spectrometer with TMS as internal standard. EI mass spectral analyses were recorded on Shimadzu Japan QP2010 S model spectrometer, and elemental analyses were carried out using Heraus CHN rapid analyzer. The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel plate using hexane and ethyl acetate. The pharmacological evaluation was carried out at the Biogenics, Hubli, Karnataka, India. The *c* log *P* values have been calculated using the Osiris molecular

property explorer software for the structural analogues of the synthesized compounds and are uncorrected. The Schiff bases 2-[4-(1-acetyl-5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)-phenyl]-4-alkyl/arylamino-5-methyl-3-oxo-2,4-dihydro-[1,2,4]-triazoles (**1a–g**) were prepared as per the reported procedure [4]. Characterization and Spectral data of synthesized compounds are tabulated in Tables 1 and 2, respectively.

3. Synthesis of 2-[4-(1-Acetyl-5-alkyl/aryl-4,5-dihydro-1*H*-pyrazol-3-yl)phenyl]-5-methyl-4{(4-amino phenyl) methylideneamino}-2,4-dihydro-3*H*-1,2,4-triazol-3-one (2a–g)

To a mixture of compound (**1a–g**, 0.50 g, 0.001 mol) and tin (0.0015 mol), concentrated HCl (3.0 mL) was added dropwise and refluxed on water bath until the solution becomes clear (approximately 30 min). The reaction mixture was then cooled and made alkaline by the addition of aqueous NaOH solution. The amino derivative thus formed was extracted with THF. The amorphous pale brown compound (**2a–g**) was obtained after evaporation of the solvent and purified using methanol/ethanol.

4. Synthesis of 3-[2-{4-(E-(-[(1-Acetyl-5-*p*-anisyl-4,5-dihydro-1*H*-pyrazol-3-yl)phenyl]-5-methyl-3-oxo-4*H*-1,2,4-triazol-yl)imino methyl}phenylhydrazinylidene]-pentane-2,4-dione (3a–g)

The amino derivative (**2a–g**, 0.001 mol) was diazotized in HCl (0.40 mL) with cold solution of sodium nitrite (0.20 g, 0.001 mol in 2.0 mL of water) during a period of 45 minutes at 0–5°C. The diazotized solution was treated with acetylacetone (0.001 mol) and sodium acetate (0.005 mol) in ethanol (10 mL) during 15 minutes and further stirred for one hour. The reaction mixture was then poured into water and the solid obtained was collected by filtration and crystallized from ethanol to get intermediate **3a–g**.

5. Synthesis of 4-{[*E*-{4-[(E)-(3,5-Dimethyl-1*H*-pyrazol-4-yl)-diazenyl] phenyl}methylidene]amino}-5-methyl-2-(1-acetyl-5,4-hydroxylphenyl-4,5-dihydro-1*H*-pyrazol-3-yl)phenyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (4a–g)

A mixture of compound **3a–g** (0.0001 mol) and hydrazine hydrate 99% (0.004 mol) in ethanol (15 mL) was refluxed on a water bath for about 8 h. The solid obtained after cooling was filtered and washed with hot ethanol to get compounds **4a–g**. Recrystallization from ethanol afforded the pale brown crystals.

6. Molecular Osiris Property Explorer [18, 19]

In view of the biological properties of azopyrazolobenzylidene derivatives as described in the introduction, it was thought of subjecting the newly synthesized molecules to molecular Osiris property explorer. Prediction of toxicity, drug likeness, and drug score by the computer programme Osiris provides the basis to avoid the experimental study of potentially harmful substances. In Osiris, toxicity risk assessment predicts mutagenicity, tumorigenicity, and irritating and reproductive effects. The Osiris property explorer is an integral part of Actelion's in-house substance registration system developed by Thomas Sander at Actelion Pharmaceuticals Limited, Switzerland. The prediction process relies on a precompiled set of structure fragment that gives rise to toxicity alerts in case they are encountered in the structure currently drawn. These fragment lists were created by rigorously shredding all compounds in the database known to be active in a certain toxicity class. During shredding, the molecule was cut at every rotatable bonds leading to a set of core fragments. These in turn were used to reconstruct all possible bigger fragments being a substructure of the original molecule. Afterwards, a substructure search process determines the occurrence frequency of any fragment within all compounds of that toxicity class.

7. Antimicrobial Assay [20, 21]

The protocol for antimicrobial activity assay was as follows.

7.1. Antibacterial Assay. Agar diffusion method was used to analyze the bacteria *S. typhi and B. pyogenes*. The standard antibiotic used was gentamycin. The media used was peptone (10 g), NaCl (10 g), and yeast extract (5 g) and agar in 1000 mL of distilled water. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37°C for 18 h. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 h old cultures (100 μL, 10^4 cfu) and spread evenly on the plate. After 20 min, the wells were filled with different concentrations of samples. The control wells were filled with gentamycin. The sample preparation was done as 20 mg sample dissolved in 1 mL of solvent (DMSO). The stock sample concentration was 20 mg/mL. The concentrations screened were 0.0625, 0.125, 0.25, 0.5, 1.0, and 2.0 mg. All the plates were incubated at 37°C for 18 h, and the diameter of the inhibition zones was noted, and MIC values are presented in Table 3.

7.2. Antifungal Assay. The fungi analyzed were *A. niger and C. albicans*. The standard antifungal used was amphotericin B. The media used was Czapek-Dox agar: composition (g/L) sucrose (30.0 g), sodium nitrate (2.0 g), K_2HPO_4 (1.0 g), $MgSO_4.7H_2O$ (0.5 g), KCl (0.5 g), $FeSO_4$ (0.01 g), and agar (20 g). Initially, the stock cultures of fungi were revived by inoculating in broth media and grown at 27°C for 48 h. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with

Table 1: Characterization data of synthesized compounds.

Comp.	Melting point (°C)	Solvent of crystallization	Yield (%)	Formula (Molecular mass)	Analysis calcd./found		
					C	H	N
2a	130-131	Methanol	60	$C_{27}H_{25}N_7O_2$ (479)	67.63 67.62	5.25 5.23	20.45 20.46
2b	140-141	Methanol	62	$C_{27}H_{24}ClN_7O_2$ (513)	63.09 63.07	4.71 4.72	19.08 19.05
2c	131-132	Methanol	63	$C_{27}H_{24}ClN_7O_2$ (513)	63.09 63.08	4.71 4.73	19.08 19.04
2d	123-124	Ethanol	60	$C_{27}H_{24}ClN_7O_2$ (513)	63.09 63.04	4.71 4.72	19.08 19.05
2e	127-128	Methanol	62	$C_{28}H_{27}N_7O_2$ (493)	68.14 68.12	5.51 5.53	19.4 19.2
2f	130-131	Ethanol	66	$C_{27}H_{25}N_7O_3$ (495)	65.44	5.09	19.79
2g	119–121	Ethanol	70	$C_{28}H_{27}N_7O_3$ (509)	65.43 66.03	5.06 5.35	19.78 19.23
3a	141-142	Ethanol	71	$C_{32}H_{30}N_8O_4$ (590)	65.07 65.08	5.12 5.13	18.97 18.96
3b	118–120	Ethanol	66	$C_{32}H_{29}ClN_8O_4$ (624)	61.49 61.50	4.68 4.69	17.93 17.90
3c	134-135	Ethanol	60	$C_{32}H_{29}ClN_8O_4$ (624)	61.49 61.51	4.68 4.68	17.93 17.92
3d	125-126	Ethanol	60	$C_{32}H_{29}ClN_8O_4$ (624)	61.49 61.49	4.68 4.67	17.93 17.89
3e	112–114	Ethanol	65	$C_{33}H_{32}N_8O_4$ (604)	65.55 65.53	5.33 5.35	18.53 18.50
3f	128-129	Ethanol	69	$C_{32}H_{30}N_8O_5$ (606)	52.36 52.33	4.4 4.1	18.47 18.49
3g	115-116	Ethanol	71	$C_{33}H_{32}N_8O_5$ (620)	63.3 63.4	5.20 5.21	18.05 18.04
4a	158–160	Ethanol	52	$C_{32}H_{32}N_{10}O_2$ (586)	65.29 65.28	5.48 5.49	23.79 23.78
4b	145-146	Ethanol	57	$C_{32}H_{31}ClN_{10}O_2$ (622)	61.68 61.65	5.01 5.02	22.48 22.47
4c	121-122	Ethanol	68	$C_{32}H_{31}ClN_{10}O_2$ (622)	61.68 61.65	5.01 5.02	22.48 22.47
4d	143-144	Ethanol	64	$C_{32}H_{31}ClN_{10}O_2$ (622)	61.68 61.65	5.01 5.02	22.48 22.47
4e	130–132	Ethanol	70	$C_{33}H_{34}N_{10}O_2$ (602)	65.76 65.75	5.69 5.68	23.24 23.27
4f	149–151	Ethanol	72	$C_{32}H_{32}N_{10}O_3$ (604)	63.56 63.57	5.33 5.34	23.16 23.14
4g	128-129	Ethanol	68	$C_{33}H_{34}N_{10}O_3$ (618)	64.06 64.04	5.54 5.55	22.64 22.65

48 h old cultures (100 μL, 10^4 cfu) and spread evenly on the plate. After 20 min, the wells were filled with different concentrations of samples. The control wells were filled with amphotericin. The sample preparation was done as 20 mg sample dissolved in 1 mL of solvent (DMSO). The stock sample concentration was 20 mg/mL. All the plates were incubated at 27°C for 48 h, and the diameter of the inhibition zones was noted, and MIC values are presented in Table 2.

8. Results and Discussion

The facile synthesis of 2-[4-(1-acetyl-5-aryl-4,5-dihydro-1*H*-pyrazol-3-yl)-phenyl]-4-amino-5-methyl-2,4-dihydro-[1,2,4]-triazol-3-one from 3-[4-(5-aryl-4,5-dihydro-1*H*-pyrazolin-3-yl)-phenyl] sydnone by two successive ring conversions has been reported from our laboratory [4]. We thought of exploiting the synthetic utility of potential amino group in these compounds. The amino group was

Table 2: Spectral data of synthesized compounds.

Comp.	IR cm^{-1}	^{1}H-NMR δ ppm	^{13}C-NMR	MS *m/e*
2a	3449–3348 (NH), 1709 and 1657 (C=O), 1603 (C=N)	2.20 (*s*, 3H, acetyl CH_3), 2.45 (*s*, 3H, CH_3), 3.67 (dd, 1H, [H_A] CH_2, J = 10.12 Hz), 4.25 (dd, 1H, [H_B] CH_2, J = 12.25 Hz), 4.89 (*m*, 1H, [H_X] CH, J_{XA} = 4 Hz, J_{XB} = 7 Hz), 5.93 (*s*, 2H, NH_2), 6.61–8.64 (*m*, 13H, Ar-H), 9.99 (*s*, 1H, N=CH)		479
2b	3450–3348 (NH), 1710, 1654 (C=O), 1605 (C=N)	2.24 (*s*, 3H, acetyl CH_3), 2.40 (*s*, 3H, CH_3), 3.70 (dd, 1H, [H_A] CH_2, J = 13.05 Hz), 4.23 (dd, 1H, [H_B] CH_2, J = 12.05 Hz), 4.90 (*m*, 1H, [H_X] CH, J_{XA} = 4.80 Hz, J_{XB} = 8.43 Hz), 7.21–8.67 (*m*, 12H, Ar-H), 9.60 (*s*, 2H, NH_2, D_2O exchangeable), 9.109 (*s*, 1H, N=CH)		513 (M+), 515 (M+2)
2c	3444–3339 (NH), 1705 and 1650 (C=O), 1600 (C=N)	2.20 (*s*, 3H, acetyl CH_3), 2.43 (*s*, 3H, CH_3), 3.68 (dd, 1H, [H_A] CH_2, J = 11.05 Hz), 4.20 (dd, 1H, [H_B] CH_2, J = 13.05 Hz), 4.2 (*m*, 1H, [H_X] CH, J_{XA} = 4.45 Hz, J_{XB} = 7.75 Hz), 7.25–8.70 (*m*, 12H, Ar-H), 9.55 (*s*, 2H, NH_2, D_2O exchangeable), 9.68 (*s*, 1H, N=CH)		513 (M+), 515 (M+2)
2d	3434–3324 (NH), 1701, 1652 (C=O), 1604 (C=N)	2.22 (*s*, 3H, acetyl CH_3), 2.45 (*s*, 3H, CH_3), 3.62 (dd, 1H, [H_A] CH_2, J = 12.03 Hz), 4.15 (dd, 1H, [H_B] CH_2, J = 11.1 Hz), 4.89 (*m*, 1H, [H_X] CH, J_{XA} = 7 Hz, J_{XB} = 4 Hz), 7.15–8.68 (*m*, 12H, Ar-H), 9.45 (*s*, 2H, NH_2, D_2O exchangeable), 9.70 (*s*, 1H, N=CH)		513 (M+), 515 (M+2)
2e	3455–3335 (NH), 1705, 1654 (C=O), 1600 (C=N)	2.25 (*s*, 3H, acetyl CH_3), 2.30 (*s*, 3H, CH_3), 2.50 (*s*, 3H, CH_3), 3.60 (dd, 1H, [H_A] CH_2, J = 11.80 Hz), 4.20 (dd, 1H, [H_B] CH_2, J = 11.1 Hz), 4.89 (*m*, 1H, [H_X] CH, J_{XA} = 7 Hz, J_{XB} = 4 Hz), 6.50–7.108 (*m*, 12H, Ar-H), 7.1 (*s*, 2H, NH_2, D_2O exchangeable), 8.11 (*s*, 1H, N=CH)		493
2f	3460 (OH), 3449–3348 (NH), 1712, 1657 (C=O), 1603 (C=N)	2.22 (*s*, 3H, acetyl CH_3), 2.47 (*s*, 3H, CH_3), 3.65 (dd, 1H, [H_A] CH_2, J = 12.10 Hz), 4.25 (dd, 1H, [H_B] CH_2, J = 12.06 Hz), 4.109 (*m*, 1H, [H_X] CH, J_{XA} = 3.32 Hz, J_{XB} = 4.32 Hz), 5.15 (*s*, 1H, OH, D_2O exchangeable), 7.25–8.65 (*m*, 12H, Ar-H), 9.50 (*s*, 2H, NH_2, D_2O exchangeable), 9.69 (*s*, 1H, N=CH)		495
2g	3439–3325 (NH), 1715, 1654 (C=O), 1605 (C=N)	2.30 (*s*, 3H, acetyl CH_3), 3.72 (*s*, 3H, $-OCH_3$), 2.47 (*s*, 3H, CH_3), 3.65 (dd, 1H, [H_A] CH_2, J = 10.10 Hz), 4.26 (dd, 1H, [H_B] CH_2, J = 12.06 Hz), 4.79 (*m*, 1H, [H_X] CH, J_{XA} = 4 Hz, J_{XB} = 7 Hz), 6.50-7.50 (*m*, 12H, Ar-H), 8.23 (*s*, 2H, NH_2, D_2O exchangeable), 8.55 (*s*, 1H, N=CH)		586
3a	1711, 1658, 1605 (C=O), 1519 (C=N)	1.97 (*s*, 3H, acetyl CH_3), 2.37 (*s*, 6H, CH_3), 2.46 (*s*, 3H, C_5–CH_3), 3.31 (dd, 1H, [H_A] CH_2, J = 11.05 Hz), 3.69 (dd, 1H, [H_B] CH_2, J = 12.06 Hz), 4.63 (*m*, 1H, [H_X] CH, J_{XA} = 4.55 Hz, J_{XB} = 8.43 Hz), 5.53 (*s*, 1H, NH (D_2O exchangeable)), 7.19–8.24 (*m*, 13H, Ar-H), 9.99 (*s*, 1H, N=CH)		590
3b	3455 (NH), 1710, 1658, 1604 (C=O), 1532 (C=N)	1.72 (*s*, 3H, acetyl CH_3), 2.30 (*s*, 6H, CH_3), 2.40 (*s*, 3H, C_5–CH_3), 3.20–3.25 (dd, 1H, [H_A] CH_2, J = 12.03 Hz), 3.71 (dd, 1H, [H_B] CH_2, J = 12.05 Hz), 4.79 (*m*, 1H, [H_X] CH, J_{XA} = 3.34 Hz, J_{XB} = 10.11 Hz), 5.55 (*s*, 1H, NH (D_2O exchangeable)), 7.25–8.30 (*m*, 12H, Ar-H), 8.64 (*s*, 1H, N=CH)		624 (M+), 626 (M+2)
3c	3445 (NH), 1715, 1656, 1600 (C=O), 1524 (C=N)	1.69 (*s*, 3H, acetyl CH_3), 2.30 (*s*, 6H, CH_3), 2.45 (*s*, 3H, C_5–CH_3), 3.24–3.27 (dd, 1H, [H_A] CH_2, J = 12.00 Hz), 3.71 (dd, 1H, [H_B] CH_2, J = 12.05 Hz), 4.79 (*m*, 1H, [H_X] CH, J_{XA} = 7 Hz, J_{XB} = 4 Hz), 5.58 (*s*, 1H, NH (D_2O exchangeable)), 6.50–7.108 (*m*, 13H, Ar-H), 8.00 (*s*, 1H, N=CH)		624 (M+), 626 (M+2)
3d	3455 (NH), 1708, 1654, 1604 (C=O), 1530 (C=N)	1.75 (*s*, 3H, acetyl CH_3), 2.35 (*s*, 6H, CH_3), 2.49 (*s*, 3H, C_5–CH_3), 3.27–3.29 (dd, 1H, [H_A] CH_2, J = 11.05 Hz), 3.75 (dd, 1H, [H_B] CH_2, J = 12.07 Hz), 4.80 (*m*, 1H, [H_X] CH, J_{XA} = 7.73 Hz, J_{XB} = 4.47 Hz), 5.57 (*s*, 1H, NH (D_2O exchangeable)), 6.55–7.75 (*m*, 13H, Ar-H), 8.13 (*s*, 1H, N=CH)		624 (M+), 626 (M+2)

Table 2: Continued.

Comp.	IR cm^{-1}	^{1}H-NMR δ ppm	^{13}C-NMR	MS *m/e*
3e	3455 (NH), 1710, 1658, 1604 (C=O), 1532 (C=N)	1.68 (*s*, 3H, acetyl CH_3), 2.36 (*s*, 6H, CH_3), 2.40 (*s*, 3H, C_5–CH_3), 2.54 (*s*, 3H, CH_3), 3.25–3.28 (dd, 1H, [H_A] CH_2, J = 13.00 Hz), 3.107 (dd, 1H, [H_B] CH_2, J = 12.07 Hz), 4.89 (*m*, 1H, [H_X] CH, J_{XA} = 5.54 Hz, J_{XB} = 8.44 Hz), 5.56 (*s*, 1H, NH (D_2O exchangeable)), 6.50–7.109 (*m*, 13H, Ar-H), 8.15 (*s*, 1H, N=CH)		604
3f	3550 (OH), 3457 (NH), 1713, 1658, 1604 (C=O), 1530 (C=N)	1.72 (*s*, 3H, acetyl CH_3), 2.37 (*s*, 6H, CH_3), 2.45 (*s*, 3H, C_5–CH_3), 3.14–3.31 (dd, 1H, [H_A] CH_2, J = 10.05 Hz), 3.72 (dd, 1H, [H_B] CH_2, J = 13.06 Hz), 4.75 (*m*, 1H, [H_X] CH, J_{XA} = 4.43 Hz, J_{XB} = 8.88 Hz), 5.53 (*s*, 1H, NH (D_2O exchangeable)), 5.60 (*s*, 1H, OH) (D_2O exchangeable), 7.20–8.25 (*m*, 13H, Ar-H), 9.00 (*s*, 1H, N=CH)		606
3g	3455 (NH), 1708, 1654, 1604 (C=O), 1532 (C=N)	1.75 (*s*, 3H, acetyl CH_3), 2.35 (*s*, 6H, CH_3), 2.49 (*s*, 3H, C_5–CH_3), 3.75 (*s*, 3H, –OCH_3), 3.27–3.29 (dd, 1H, [H_A] CH_2, J = 12.05 Hz), 3.75 (dd, 1H, [H_B] CH_2, J = 12.07 Hz), 4.80 (*m*, 1H, [H_X] CH, J_{XA} = 4.80 Hz, J_{XB} = 8.48 Hz), 5.55 (*s*, 1H, NH (D_2O exchangeable)), 6.55–7.75 (*m*, 13H, Ar-H), 8.15 (*s*, 1H, N=CH)		620
4a	3426 (NH), 1704, 1617 (C=O), 1519 (C=N)	1.18 (*s*, 1H, NH), 1.58 (*s*, 3H, acetyl CH_3), 2.29 (*s*, 3H, CH_3), 2.36 (*s*, 3H, CH_3), 2.45 (*s*, 3H, C_5–CH_3), 3.63 (dd, 1H, [H_A] CH_2, J = 11.03 Hz), 3.73 (dd, 1H, [H_B] CH_2, J = 12.00 Hz), 4.24 (*s*, 6H, CH_3), 5.51 (*m*, 1H, [H_X] CH, J_{XA} = 4.40 Hz, J_{XB} = 11.00 Hz), 7.19–7.96 (*m*, 13H, Ar-H), 7.99 (*s*, 1H, C=NH)	10.73, 12.63, 21.95, 44.51, 60.57, 107.83, 119.80, 126.39, 129.60, 129.06, 130.12, 130.82, 139.46, 142.53, 151.29, 154.32, 155.87, 169.21	586
4b	3430 (NH), 1707, 1620 (C=O), 1594 (C=N)	1.16 (*s*, 1H, NH), 2.00 (*s*, 3H, acetyl CH_3), 2.31 (*s*, 3H, CH_3), 2.40 (*s*, 3H, CH_3), 2.50 (*s*, 3H, C_5–CH_3), 3.70 (dd, 1H, [H_A] CH_2, J = 12.03 Hz), 3.107 (dd, 1H, [H_B] CH_2, J = 11.00 Hz), 4.30 (*s*, 6H, CH_3), 5.55 (*m*, 1H, [H_X] CH, J_{XA} = 4.80 Hz, J_{XB} = 11.42 Hz), 7.25–8.00 (*m*, 12H, Ar-H), 8.05 (*s*, 1H, C=NH)	10.56, 11.72, 21.44, 45.81, 60.51, 107.69, 119.03, 127.73, 29.72, 130.01, 131.12, 131.82, 139.92, 142.35, 150.58, 154.43, 155.62, 169.39	622 (M+), 624 (M+2)
4c	3420 (NH), 1710, 1625 (C=O), 1593 (C=N)	1.19 (*s*, 1H, NH), 2.05 (*s*, 3H, acetyl CH_3), 2.34 (*s*, 3H, CH_3), 2.43 (*s*, 3H, CH_3), 2.52 (*s*, 3H, C_5–CH_3), 3.71 (dd, 1H, [H_A] CH_2, J = 14.03 Hz), 3.107 (dd, 1H, [H_B] CH_2, J = 12.05 Hz), 4.30 (*s*, 6H, CH_3), 5.30 (*m*, 1H, [H_X] CH, J_{XA} = 4.5 Hz, J_{XB} = 11.00 Hz), 7.30–7.12 (*m*, 12H, Ar-H) 8.00 (*s*, 1H, C=NH)	9.13, 11.79, 21.69, 42.11, 59.48, 105.39, 117.51, 125.39, 127.16, 127.76, 128.23, 128.62, 137.03, 142.35, 150.48, 153.32, 154.69, 167.36	622 (M+), 624 (M+2)
4d	3435 (NH), 1710, 1627 (C=O), 1560 (C=N)	1.16 (*s*, 1H, NH), 2.07 (*s*, 3H, acetyl CH_3), 2.32 (*s*, 3H, CH_3), 2.42 (*s*, 3H, CH_3), 2.51 (*s*, 3H, C_5–CH_3), 3.109 (dd, 1H, [H_A] CH_2, J = 11.03 Hz), 3.80 (dd, 1H, [H_B] CH_2, J = 12.00 Hz), 4.35 (*s*, 6H, CH_3), 5.56 (*m*, 1H, [H_X] CH, J_{XA} = 3.33 Hz, J_{XB} = 9.89 Hz), 7.24–8.05 (*m*, 12H, Ar-H) 8.10 (*s*, 1H, C=NH)	8.79, 11.63, 22.41, 41.14, 58.44, 106. 43, 118.51, 124.82, 126.82, 127.62, 128.88, 128.96, 142.71, 151.51, 153.53, 154.46, 167.91	622 (M+), 624 (M+2)
4e	3420 (NH), 1710, 1625 (C=O), 1593 (C=N)	1.19 (*s*, 1H, NH), 2.05 (*s*, 3H, acetyl CH_3), 2.30 (*s*, 3H, CH_3), 2.43 (*s*, 3H, CH_3), 2.47 (*s*, 3H, C_5–CH_3), 3.71 (dd, 1H, [H_A] CH_2, J = 14.03 Hz), 3.107 (dd, 1H, [H_B] CH_2, J = 13.05 Hz), 4.30 (*s*, 6H, CH_3), 5.30 (*m*, 1H, [H_X] CH, J_{XA} = 4.00 Hz, J_{XB} = 9 Hz), 7.30–7.92 (*m*, 12H, Ar-H), 7.99 (*s*, 1H, C=NH)	9.12, 11.81, 22.59, 41.12, 58.91, 106.34, 118.51, 124.32, 126.93, 127.71, 128.42, 128.69, 143.01, 151.01, 153.35, 154.79, 167.66	602
4f	3510 (OH), 3420 (NH), 1715, 1623 (C=O), 152 (C=N)	1.71 (*s*, 1H, NH), 2.10 (*s*, 3H, acetyl CH_3), 2.35 (*s*, 3H, CH_3), 2.45 (*s*, 3H, CH_3), 2.49 (*s*, 3H, C_5–CH_3), 3.71 (dd, 1H, [H_A] CH_2, J = 12.05 Hz), 3.108 (dd, 1H, [H_B] CH_2, J = 15.05 Hz), 4.35 (*s*, 6H, CH_3), 5.32 (*m*, 1H, [H_X] CH, J_{XA} = 4.43 Hz, J_{XB} = 8.50 Hz), 5.50 (*s*, 1H, OH), 7.35–7.1 (*m*, 12H, Ar-H), 8.11 (*s*, 1H, C=NH)	9.15, 11.83, 22.58,14.12, 59.96, 107.39, 118.75, 125.39, 126.83, 127.91, 128.42, 128.84, 144.61, 151.48, 153.84, 154.86, 168.69	604
4g	3437 (NH), 1708, 1627 (C=O), 1567 (C=N)	1.67 (*s*, 1H, NH), 2.08 (*s*, 3H, acetyl CH_3), 2.35 (*s*, 3H, CH_3), 2.47 (*s*, 3H, CH_3), 2.52 (*s*, 3H, C_5–CH_3), 3.73 (*s*, 3H, –OCH_3), 3.80 (dd, 1H, [H_A] CH_2, J = 14.03 Hz), 3.2 (dd, 1H, [H_B] CH_2, J = 10.00 Hz), 4.37 (*s*, 6H, CH_3), 5.56 (*m*, 1H, [H_X] CH, J_{XA} = 4.00 Hz, J_{XB} = 11.00 Hz), 7.25–8.07 (*m*, 12H, Ar-H) 8.10 (*s*, 1H, C=NH)	8.47, 12.38, 23.82, 42.85, 59.36, 106.28, 118.84, 126.92, 127.84, 128.47, 129.02, 129.96, 143.44, 151.86, 154.95, 155.79, 169.61	618

Table 3: Molecular Osiris property of compounds **4a–g**.

Entry no.	*c* log *P*	Mol Wt	Drug likeliness	Drug score
4a	6.08	580	−1.34	0.05
4b	6.69	620	−1.65	0.04
4c	6.69	620	−2.10	0.04
4d	6.69	620	−0.44	0.04
4e	6.40	600	−3.14	0.04
4f	5.109	602	−1.108	0.04
4g	5.4	616	−1.84	0.04

unreactive towards reagents like α-halogen esters, KCNS, nitrous acid, and so forth, indicating its weak nucleophilic character, which is due to strong electron-withdrawing 1,2,4-triazolin-2-one ring. In an attempt to prepare the azopyrazolobenzylidene derivatives, we thought of using the 4-(4-nitrobenzylideneamino)-2-(4-(1-acetyl-4,5-dihydro-5-aryl-1*H*-pyrazol-3-yl)phenyl)-5-methyl-2*H*-1,2,4-triazol-3(4*H*)-one **1a–g** as starting material. The compounds **1a–g** on reduction with Sn/HCl gave the compound **2a–g**, and further reaction with sodium nitrite, sodium acetate, and acetylacetone yielded the 2,4-dione **3a–g** (*Japp-Klingemann reaction*). The reaction of compound **3a–g** with hydrazine hydrate afforded azopyrazolobenzylidene derivatives **4a–g** (Figure 1). The mechanism of conversion of compounds **1a–g** to **4a–g** is presented in Figure 2.

The mechanism involves diazotization of compound **2a–g** to give diazonium salt which on coupling with enolate form of acetylacetone gave diazo compound. Hydrazine hydrate was made to attack two carbonyl groups of the diazo compound nucleophilically, and subsequent dehydration of the formed intermediate gave final compounds **4a–g**.

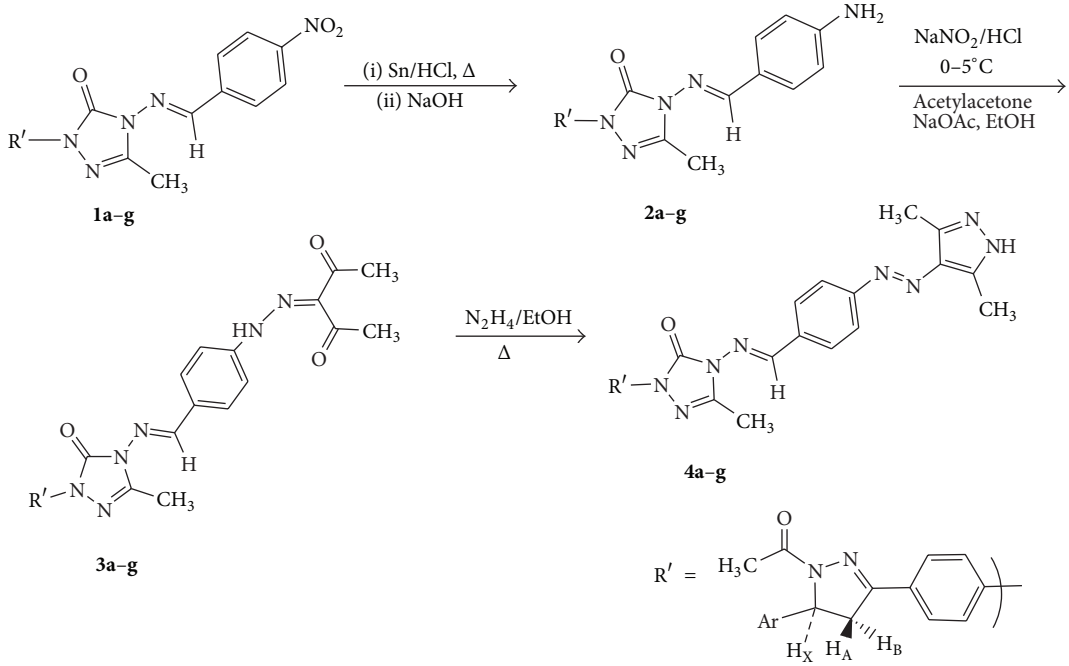
The Schiff base of 2-[4-(1-acetyl-5-nitrophenyl-4,5-dihydro-1*H*-pyrazol-3-yl)-phenyl]-4-amino-5-methyl-2,4-dihydro-[1,2,4]-triazol-3-one **1a–g** has been utilized as useful precursor for the synthesis of pyrazole derivatives. Exploitation of structure activity relationship (SAR) studies of such combination of heterocycles could lead to the development of congener with more therapeutic index than those of parent compounds. There is a considerable interest in chemotherapeutic activity of triazole and pyrazole derivatives, and hence, interesting pharmacological properties have been claimed. The synthesis of pyrazole built on the *para* position of phenyl ring attached to sydnone is reported from this laboratory [4]. These observations and the ease with which the compounds having two pyrazole and one 1,2,4-triazole ring encouraged us to explore present work. Though many pyrazole derivatives have been reported, it is for the first time that a molecule having two pyrazoles spaced with 1,2,4-triazole were synthesized starting from N-arylsydnones as synthons.

The IR spectra of intermediate amino compounds **2a–g** showed bands for the asymmetric and symmetric stretching vibrations of NH_2 in the range of 3455–3434 cm^{-1} and 3348–3324 cm^{-1}, respectively. The carbonyl group of the 1,2,4-triazolin-2-one ring appeared as a sharp band at 1715–1705 cm^{-1} and that of acetyl group appeared around 1657–1650 cm^{-1}. The C=N group showed a band at 1600–1653 cm^{-1}. Compound **2e** showed a band at 3455 cm^{-1} for OH stretching. The IR spectra of diones **3a–g** showed a broad band at 3455–3439 cm^{-1} due to NH vibrations. The carbonyl group of the 2,4-dione moiety appeared at 1658–1652 cm^{-1}, while that of the 1,2,4-triazolin-2-one ring appeared at 1715–1710 cm^{-1}. Also, carbonyl of acetyl group appeared around 1600–1604 cm^{-1}. The C=N group showed a band around 1519–1532 cm^{-1}. Compound **2e** showed a band at 3550 cm^{-1} for OH group. The IR spectra of the compounds **4a–g** showed a broadband at 3437–3426 cm^{-1} due to NH vibrations. The IR spectra showed C=O group of 1,2,4-triazolinone ring at 1715–1704 cm^{-1} and C=O of acetyl group appeared at 1627–1620 cm^{-1}. The absence of the C=O at 1609 cm^{-1} evidences the formation of the pyrazole ring. The C=N group showed a band at 1594–1519 cm^{-1}.

The H_A and H_B protons appeared as doublet due to geminal and vicinal coupling. These H_A and H_B differ in coupling with the Hx, and hence, they are also anisogamous. The H_A proton appears as doublet of doublet in the range δ 3.109–3.31 ppm. The H_B also appeared as doublet of doublet at δ 3.72–4.25 ppm. where J_{BA} is in the range 12.25–17.23 Hz. The Hx always appeared as four-line spectrum in the range δ 3.72–5.56 ppm. The ^{1}H NMR spectra of these compounds **2a–g** showed a singlet at δ 8.23–9.50 ppm. (D_2O exchangeable) for the NH_2 protons. The singlets at δ 2.30–2.40 ppm. and 2.45–2.47 ppm. were observed for methyl protons of triazolinone (C_5–CH_3) and that of acetyl group respectively. Another singlet was observed around δ 8.55–9.99 ppm. was due to the imine proton and the aromatic protons were resonated in the range δ 7.19–8.69 ppm. The ^{1}H NMR spectra of the compounds **3a–g** showed a singlet for acetyl CH_3 at δ 2.30–2.37 ppm. and a singlet at δ 1.68–1.97 ppm. for the two CH_3 groups of the 2,4-dione; the C_5–CH_3 appeared at δ2.45–2.49 ppm., and the imine –CH appeared at δ 8.15–9.99 ppm. The NH proton was observed at δ 5.53–5.57 ppm. (D_2O exchangeable). The ^{1}H NMR spectra of compounds **4a–g** showed a singlet for acetyl CH_3 at δ 1.58–2.08 ppm. and the two CH_3 protons of the pyrazole ring as two singlets at δ 2.29–2.35 ppm. and δ 2.36–2.47 ppm. The C_5–CH_3 also appeared at δ 2.45–2.52 ppm. as singlet. The compounds **2e**, **3e**, and **4e** showed a singlet for three protons of C_5–CH_3 in the range 2.29–2.37 ppm. The compounds **2f**, **3f**, and **4f** showed a singlet for –OH at 5.15, 5.60, and 5.50 ppm., respectively. Also, singlets for –OCH_3 at δ 3.72, 3.75, and 3.11 ppm. were observed in the compounds **2 g**, **3 g**, and **4 g**.

9. Biological Activity

Further, in the present investigation, attention has also been diverted on structure activity relationship (SAR) by way of computational studies applying Osiris property explorer and analyzed for their drug score. The synthesized title compounds were also screened for antibacterial and antifungal activities and calculated the IC 50 values (graphs are provided in the Supplementary file) (See Supplementary Material available online at http://dx.doi.org/10.1155/2013/909706).



a = Ar = phenyl, **b** = Ar = *o*-chlorophenyl,
c = Ar = *m*-chlorophenyl, **d** = Ar = *p*-chlorophenyl,
e = Ar = *p*-tolyl, **f** = Ar = *p*-hydroxyphenyl,
g = Ar = *p*-anisyl

Figure 1

9.1. Molecular Osiris Property Explorer. It was possible to predict the biological activity of all the synthesized compounds in terms of their toxicity by employing toxicity risk assessment through Osiris property explorer which calculates on-the-fly various drug-relevant properties whenever a structure is valid. Prediction results are valued and color coded [22, 23]. Properties with high risks of undesired effects like mutagenicity or a poor intestinal absorption are shown in red, whereas a green color indicates drug-conform behavior. In the latter study, all the compounds were evaluated for the presence of various toxicity parameters like mutagenicity, tumorigenicity, reproductive affective effects, and irritation. In the Osiris explorer tool, the drug score is calculated based on the combination of toxicity risks (mutagenicity, tumorigenicity, irritation, and reproduction), drug likeness, and some physicochemical parameters such as *c* log *P*, log *S* (solubility), and molecular weight in one handy value than may be used to judge the compound's overall potential to qualify for a drug (based on the Lipinski rule of five). The title compounds do not violate the Lipinski rule and they fall well in the range. The target compounds showed moderate to good drug score (0.12–0.36) that revealed their potential as safe lead compounds. The drug likeness value ranged from −3.14 to −0.44, whereas the drug score ranged from 0.04 to 0.05. The *chloro* substituent in the compounds **4b**, **4c**, and **4d** was shifted to different positions (*ortho*, *para*, and *meta*) to study the change on the positional effect of substituent. Interestingly, these compounds showed almost the same drug score which indicates that there is no positional effect on these compounds. The compound **4g** which has *p*-anisyl group showed less drug likeness and also the drug score was less. The compounds **4e** and **4f** which have electron donating groups show good drug score as depicted in Table 3. The compound **4a** has also shown diversified effects based on drug likeness and drug score, but *c* log *P* values are well within the range as mentioned by the rule.

9.2. Antimicrobial Activity. MIC values for the *in vitro* antibacterial studies of the compounds **4a–g** and the standard are represented in Table 4 which fall at 09.00–21.00 mg/mL for antibacterial, and antifungal lies in the range 24.00–72.00 g/mL. The antibacterial activity of the compounds **4a**, **4e**, **4f**, and **4g** against *S. typhi* and *B. pyogenes* showed excellent potencies compared to standard drugs gentamycin. The compounds **4a** and **4g** (*phenyl, p-anisyl*) have shown excellent potency against *S. typhi*. The compounds **4a** and **4f** (*phenyl, p-hydroxy*) have shown excellent potency against *B. pyogenes*. The compound **4e** has shown good potency against both the bacterial strains tested. The antibacterial activity may be attributed towards the electron donating groups attached to the phenyl ring. The compounds **4b**, **4c**, and **4d** have shown moderate potencies towards antibacterial activity. The compounds **4b**, **4c**, and **4d** have good results towards antifungal activity. The compound **4d** (*p-chloro*) has shown excellent potency against both the fungal strains *A. niger* and *C. albicans* compared to standard drug amphotericin. The other two compounds **4b** and **4c** have shown good results against both strains tested. The antifungal activity may be due

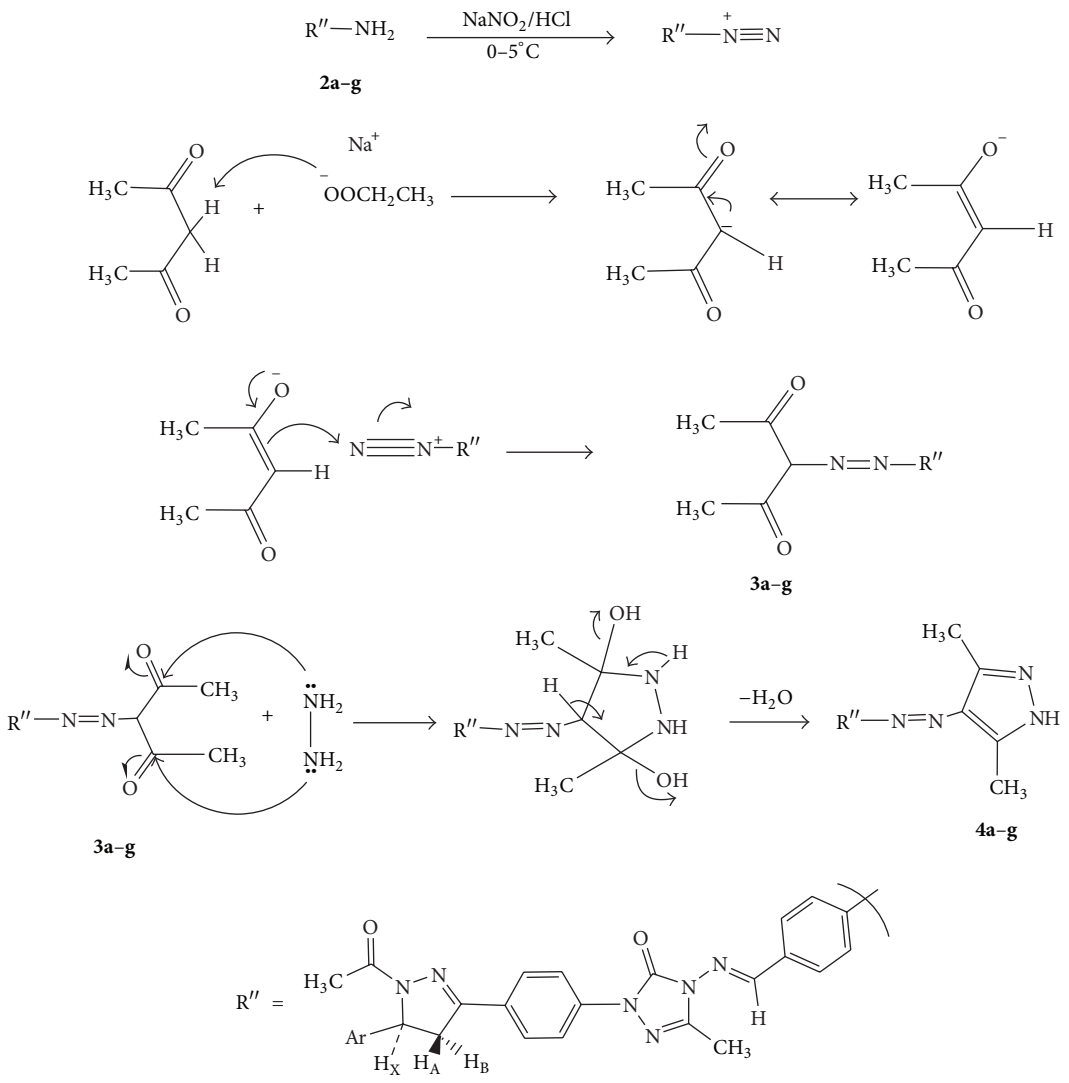


Figure 2

to the presence of halogen group on the phenyl ring. All other compounds have shown moderate activity against the fungal strains. From the results, it is apparent that among the title compounds, all of them, have shown excellent to moderate activity at 9.00–21.00 mg/mL and 24.00–72.00 mg/mL against all the screened bacteria and fungi. The results are in well agreement with the drug scores obtained from the Osiris property explorer. MIC values of all the triazole derivates **4a–g** are excellent and promising because of the presence two pyrazole rings in a single moiety making them more potent towards the bacterial and fungal strains.

The IC 50 values of antimicrobial activity revealed that the compounds (**4f** and **4g**) and (**4a** and **4b**) have shown very good antifungal activity against *A. niger* and *C. albicans*, respectively, whereas the compounds (**4b** and **4g**) and **4c** are potent against *S. typhi* and *B. pyogenes*, respectively.

Table 4: Antimicrobial activities of the compounds (MIC, mg/mL) **4a–g**.

Entry no.	*S. typhii*	*B. pyogenes*	*A. niger*	*C. albicans*
4a	18.0	21.0	40.0	24.0
4b	12.0	10.0	65.0	42.0
4c	09.00	13.0	60.0	40.0
4d	14.0	09.00	72.0	43.0
4e	15.0	18.0	44.0	27.0
4f	16.0	22.0	38.0	25.0
4g	21.0	19.0	43.0	33.0
Gentamycin	25	25	—	—
Amphotericin	—	—	100	50
Control	DMSO	DMSO	DMSO	DMSO

10. Conclusion

In conclusion, we have developed a simple and efficient method for the synthesis of novel pyrazolyl triazole derivatives through reduction of nitro group and further reacting with acetyl acetone and hydrazine hydrate. We also believe that the procedural simplicity, the efficiency, and the easy accessibility of the reaction partners give access to an array of heterocyclic frameworks. The results of the antimicrobial activity (MIC and IC 50) revealed that all of the 7 compounds showed excellent to moderate inhibition against the bacteria and fungi screened.

Conflict of Interests

The authors confirm that this paper has no conflict of interests.

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