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ARTICLE

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Design, synthesis of tri-substituted pyrazole derivatives as promising antimicrobial agents and investigation of structure activity relationships

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Abstract

The major diseases spread in the environment only because of microbes. Even, intensive care units in the hospitals are polluted by microorganisms, particularly, Gram-positive bacteria. Although many antibiotics are existed, there is a need to develop up to date microbial-resistant agents. Hence, the current study aimed to develop prominent antimicrobial drug-related compounds. Thus, a novel series of tri-substituted analogs and their intermediates were synthesized. In addition, total new compounds were screened for their antimicrobial assay and identified as the most efficient biologically active compounds. Moreover, minimal inhibitory antimicrobial activity and appropriate structure activity relationships were investigated. From the results it was observed that, viability of Gram-positive bacteria was most powerfully affected by all active compounds. Finally, this research demonstrated that, these biologically energetic amalgams can be utilized for further preclinical studies with the ambition of standing unique inventive drugs.

1 | INTRODUCTION

There are many bacterial and fungal infections existed in the world. Consequently, persons are exposed to illnesses caused by microbial. Nowadays, in infirmaries, the bacterial infections predominantly Gram-positive bacteria (GPB) contagions remain the critical problem.[1,2] Interestingly, majority of microbes are isolated from intensive care units, particularly, GPB isolation percentage is more than other bacteria.[3] In most circumstances, the protected system of healthy humans can manage pathogenic attacks powerfully. But those systems only control the damage of cells, but not cure. Thus, use of antibiotics is raising and parallel to this, antibacterial opposition has been increasing to hazardous levels in all parts of the world.[4,5] On the other hand, the frequency of fungal poisons has progressively increased from the last 20 years and presents a risky threat to people health, specifically in immunocompromised humans, like those experiencing organ exchanges or antitumor chemotherapy and acquired immunodeficiency syndrome patients.[6–8] In current years, the investigation has been dedicated on the enlargement of innovative antimicrobial stuffs, those can act over

structure design and novel targets, overcoming the difficult of antibiotic resistance.[9–12]

Azole derivatives particularly, pyrazoles retain pharmacological and biotic properties.[13] Mostly in the farming field, pyrazole core is generally measured as a vital active part of some fungicidal,[14,15] insecticidal,[16] antiviral,[17] and acaricidal molecules.[18] For example, marketable pesticide Tebufenpyrad and Tolfenpyrad[19] have pyrazole moiety as a basic core. In addition, fungicide Pyraclostrobin[20] is contained a pyrazole component. A short time ago, Dai et al reported a novel series of bispyrazole derivatives displayed worthy antiproliferative activity.[21] Besides, pyrazole-contained drugs are present in top-selling medicines, displayed antidepressive, antihyperglycemic, antispasmodic, antibacterial and anti-inflammatory actions.[22] Well-known samples are Celecoxib[23] (Celebrex) and Sildenafil[24] (Viagra). Products of pyrazoles have been exposed to possess diverse properties including, but not restricted to, being antitumor,[25] antidiabitic,[26] anti-inflammatory,[27] antiarrhythmic,[28] monoamine oxidase inhibiting,[29] sPLA2-inhibitory,[30] antipyretic,[31] analgesic,[32] anticonvulsant,[33] and antimicrobial.[34] In fact, preparation method equally represents a pivotal role to discover the biologically active pyrazoles and their belongings. There are different synthetic approaches reported.[35–39] All those showed their unique advantageous.

Based on the prior information and our continued interest to develop biologically active substances[40–43]; we have tried synthesizing pyrazole derivatives to achieve potent antimicrobial agents. All the compounds were characterized by spectroscopic techniques. Total outcome compounds were evaluated for their antibacterial and antifungal assays. In addition, structure activity relationships are also discussed.

2 | EXPERIMENTAL

2.1 | General

Total primary components and reagent were pure and commercially available. For ^{1}H NMR, 400 MHz and for ^{13}C NMR 100 MHz were used. Dimethyl sulfoxide and chloroform deuterated mixed solvent was used to record both nuclear magnetic resonance (NMR) spectra's. Tetramethylsilane (TMS) as a reference sample. Melting point statistics were distinguished with micro melting point operator and were uncorrected. High-resolution mass spectrometry (HRMS) information was calculated with the help of electrospray ionization. The intermediate one (**5**) was prepared according to the literature procedure.[44,45]

2.1.1 | Synthetic method of compound 6

Similar volume of intermediate compound **5** (1 mmol) and semicarbazide (1 mmol) were taken in a round bottom flask which already contained methanol (5 mL), NaOH (1.5 mmol) and refluxed for 5 to 8 hours. After completion of the product formation (checked by thin-layer chromatography), the reaction crud was transferred onto crushed ice. As a result, solid on the bottom of the water, which was collected by filtration and purified by recrystallization from 2-propanol resulted in compound **6**.

3-(1-Oxo-1H-isochromen-3-yl)-5-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (**6**): Pale yellow solid; yield 85%; mp 173-175°C; ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6): δ 3.18 (dd, H_X, 1H, J_{MX} = 12.0 Hz, J_{AX} = 6.6 Hz), 3.88 (dd, H_M, 1H, J_{MX} = 12.0 Hz, J_{AM} = 14.7 Hz), 5.28 (dd, H_A, 1H, J_{AX} = 6.6 Hz, J_{AM} = 14.7 Hz), 6.91-7.81 (m, 10H, Ar—H and C_4—H), 8.66 (bs, 2H, NH_2) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 43.4 (C−4), 67.6 (C−5), 121.5, 122.9, 125.2, 127.4, 128.2, 129.2, 131.4, 132.2, 134.1, 136.5, 140.2, 143.2 (aromatic carbons), 156.1 (C−3), 178.3 (C═O), 182.3 (C═O—NH_2) ppm; HRMS: *m/z* Calcd. for $C_{19}H_{16}N_3O_3$ $(M + H)^+$ 334.1192; Found 334.1190.

2.1.2 | Dehydrogenation method belongs to the compound 7

In xylene (7 mL), intermediate **6** (1 mmol) and chloranil (2 mmol) were dissolved. Then, the set up was kept to reflux for 24 hours. After the product formation, the crud was washed with 5% sodium hydroxide solution followed by the separation of organic part, dried by using anhydrous sodium sulfate and organic solvent was eliminated in vacuo. The obtained solid was purified by using 2-propanol solvent.

3-(1-Oxo-1H-isochromen-3-yl)-5-phenyl-1H-pyrazole-1-carboxamide (**7**): Light yellow solid; yield 81%; mp 149-151°C; ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6): 7.32-7.71 (m, 11H, Ar—H and $C_{4'}$—H), 8.51 (bs, 2H, NH_2) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 121.6, 123.4, 124.5, 125.1, 126.3, 128.2, 129.0, 130.1, 133.3, 135.2, 136.8, 139.0, 142.1, 144.8 (aromatic carbons), 155.3 (C−3), 176.4 (C═O), 181.2 (C═O—NH_2) ppm; HRMS: *m/z* Calcd. for $C_{19}H_{14}N_3O_3$ $(M + H)^+$ 332.1035; Found 332.1031.

2.1.3 | Reaction method for the compounds 9(a-l)

Condensation reaction was done between the compound **7** (1 mmol) and araldehydes **8**(**a**-**l**) (1 mmol) in acetic acid (7 mL) under refluxtion for 8 to 10 hours. Then, the crude was poured into crushed ice and the solid was separated

by filtration followed by washing with dilute hydrochloride solution. The resultant solid was recrystallized from propanol.

(E)-N-Benzylidene-3-(1-oxo-1H-isochromen-3-yl)-5-phenyl-1H-pyrazole-1-carboxamide (**9a**): Light yellow solid; yield 82%; mp 234-236°C; ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6): δ 6.69-7.84 (m, 16H, Ar—H), 8.91 (s, 1H, HC═NCO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 110.3, 112.9, 117.6, 120.1, 123.6, 124.4, 126.4, 127.7, 128.3, 128.8, 129.6, 129.9, 131.2, 134.5, 141.6, 143.4, 144.4, 146.5, 148.0 (aromatic carbons), 162.6 (HC═NH), 171.1 (CO—O), 180.4 (N—CO—N) ppm; HRMS: *m/z* Calcd. for $C_{26}H_{18}N_3O_3$ $(M + H)^+$ 420.1348; Found 420.1346.

(E)-N-(4-Chlorobenzylidene)-3-(1-oxo-1H-isochromen-3-yl)-5-phenyl-1H-pyrazole-1-carboxamide (**9b**): Light yellow solid; yield 84%; mp 197-199°C; ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6): δ 6.83-7.88 (m, 15H, Ar—H), 8.97 (s, 1H, HC═NCO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 110.2, 112.4, 118.2, 120.5, 122.9, 123.7, 126.3, 127.3, 127.9, 128.6, 129.2, 130.1, 131.4, 135.0, 142.1, 143.6, 145.6, 150.1, 151.5 (aromatic carbons), 163.2 (HC═NH), 170.3 (CO—O), 180.9 (N—CO—N) ppm; HRMS: *m/z* Calcd. for $C_{26}H_{17}ClN_3O_3$ $(M + H)^+$ 454.0958; Found 454.0954.

(E)-N-(4-Methylbenzylidene)-3-(1-oxo-1H-isochromen-3-yl)-5-phenyl-1H-pyrazole-1-carboxamide (**9c**): Pale yellow solid; yield 78%; mp 221-223°C; ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6): δ 2.23 (s, 3H, CH_3), 6.74-7.88 (m, 15H, Ar—H), 8.83 (s, 1H, HC═NCO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 19.6 (CH_3), 110.1, 112.4, 118.6, 120.2, 121.5, 123.4, 125.1, 126.3, 127.4, 128.3, 129.7, 131.4, 132.7, 134.1, 137.2, 140.3, 142.4, 145.6, 146.9 (aromatic carbons), 162.6 (HC═NH), 170.6 (CO—O), 180.4 (N—CO—N) ppm; HRMS: *m/z* Calcd. for $C_{27}H_{20}N_3O_3$ $(M + H)^+$ 434.1505; Found 434.1502.

(E)-N-(2-Aminobenzylidene)-3-(1-oxo-1H-isochromen-3-yl)-5-phenyl-1H-pyrazole-1-carboxamide (**9d**): Light yellow solid; yield 72%; mp 184-186°C; ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6): δ 6.73-7.79 (m, 15H, Ar—H), 8.85 (s, 1H, HC═NCO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 109.3, 114.5, 116.8, 121.2, 122.7, 123.8, 124.7, 126.5, 127.0, 128.1, 129.5, 130.0, 130.9, 131.4, 133.7, 135.4, 140.2, 143.4, 145.1, 147.6, 148.4 (aromatic carbons), 161.5 (HC═NH), 169.4 (CO—O), 180.4 (N—CO—N) ppm; HRMS: *m/z* Calcd. for $C_{26}H_{19}N_4O_3$ $(M + H)^+$ 435.1457; Found 435.1452.

(E)-N-(4-Hydroxybenzylidene)-3-(1-oxo-1H-isochromen-3-yl)-5-phenyl-1H-pyrazole-1-carboxamide (**9e**): Pale yellow solid; yield 71%; mp 227-229°C; ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6): δ 6.81-7.73 (m, 15H, Ar—H), 8.93 (s, 1H, HC═NCO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 111.4, 114.3, 119.5, 121.3, 122.5, 124.1, 125.2, 126.3, 127.5, 128.3, 129.0, 131.3, 132.2, 134.6, 140.3, 142.9, 144.1, 146.2, 147.8 (aromatic carbons), 161.1 (HC═NH), 172.5 (CO—O), 181.4 (N—CO—N) ppm; HRMS: *m/z* Calcd. for $C_{26}H_{18}N_3O_4$ $(M + H)^+$ 436.1297; Found 436.1296.

(E)-N-(4-Nitrobenzylidene)-3-(1-oxo-1H-isochromen-3-yl)-5-phenyl-1H-pyrazole-1-carboxamide (**9f**): Pale yellow solid; yield 80%; mp 190-192°C; ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6): δ 6.91-7.89 (m, 15H, Ar—H), 8.90 (s, 1H, HC═NCO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 111.3, 115.7, 118.0, 120.4, 121.3, 122.6, 124.1, 125.7, 126.9, 128.2, 129.0, 130.5, 131.1, 133.5, 140.2, 143.6, 146.9, 148.4, 150.2 (aromatic carbons), 162.9 (HC═NH), 171.2 (CO—O), 182.3 (N—CO—N) ppm; HRMS: *m/z* Calcd. for $C_{26}H_{17}N_4O_5$ $(M + H)^+$ 465.1199; Found 465.1195.

(E)-N-(2-Methylbenzylidene)-3-(1-oxo-1H-isochromen-3-yl)-5-phenyl-1H-pyrazole-1-carboxamide (**9g**): Pale yellow solid; yield 72%; mp 202-204°C; ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6): δ 2.21 (s, 3H, CH_3), 6.71-7.84 (m, 15H, Ar—H), 8.80 (s, 1H, HC═NCO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 19.6 (CH_3), 115.2, 117.1, 120.0, 121.3, 122.9, 123.3, 125.2, 126.1, 126.9, 127.5, 129.1, 130.4, 131.9, 132.6, 133.0, 136.1, 140.2, 142.2, 144.7, 146.0, 147.2 (aromatic carbons), 163.4 (HC═NH), 170.2 (CO—O), 181.5 (N—CO—N) ppm; HRMS: *m/z* Calcd. for $C_{27}H_{20}N_3O_3$ $(M + H)^+$ 434.1505; Found 434.1503.

(E)-N-(4-Aminobenzylidene)-3-(1-oxo-1H-isochromen-3-yl)-5-phenyl-1H-pyrazole-1-carboxamide (**9h**): Light yellow solid; yield 74%; mp 206-208°C; ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6): δ 6.70-7.82 (m, 15H, Ar—H), 8.86 (s, 1H, HC═NCO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 113.1, 116.4, 119.0, 120.4, 122.4, 123.7, 125.1, 126.4, 127.8, 128.6, 129.2, 132.2, 133.6, 135.2, 142.6, 143.1, 145.7, 147.1, 148.9 (aromatic carbons), 163.4 (HC═NH), 172.3 (CO—O), 180.1 (N—CO—N) ppm; HRMS: *m/z* Calcd. for $C_{26}H_{19}N_4O_3$ $(M + H)^+$ 435.1457; Found 435.1456.

(E)-N-(2-Methoxybenzylidene)-3-(1-oxo-1H-isochromen-3-yl)-5-phenyl-1H-pyrazole-1-carboxamide (**9i**): Pale yellow solid; yield 70%; mp 189-191°C; ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6): δ 3.53 (s, 3H, OCH_3), 6.76-7.82 (m, 15H, Ar—H), 8.85 (s, 1H, HC═NCO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 59.2 (OCH_3), 110.2, 115.6, 119.2, 120.7, 121.2, 123.4, 124.5, 125.5, 126.1, 127.6, 128.4, 129.8, 130.2, 131.2, 132.6, 134.1, 137.4, 140.3, 143.5, 144.9, 146.0 (aromatic carbons), 160.4 (HC═NH), 168.2 (CO—O), 180.3 (N—CO—N) ppm; HRMS: *m/z* Calcd. for $C_{27}H_{20}N_3O_4$ $(M + H)^+$ 450.1454; Found 450.1451.

(E)-N-(2-Hydroxybenzylidene)-3-(1-oxo-1H-isochromen-3-yl)-5-phenyl-1H-pyrazole-1-carboxamide (**9j**): Pale yellow solid; yield 71%; mp 194-196°C; ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6): δ 6.90-7.85 (m, 15H, Ar—H), 8.89 (s, 1H, HC═NCO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 109.2, 116.1, 118.2, 120.4, 123.2, 124.2, 125.1, 126.6, 127.2, 128.5, 129.2, 131.4, 132.5, 133.9, 135.4, 137.2, 141.3, 144.8, 146.1, 147.4, 149.2 (aromatic carbons), 160.4 (HC═NH),

170.6 (C̲O—O), 182.2 (N—C̲O—N) ppm; HRMS: *m/z* Calcd. for $C_{26}H_{18}N_3O_4$ $(M + H)^+$ 436.1297; Found 436.1294.

(E)-N-(4-Methoxybenzylidene)-3-(1-oxo-1H-isochromen-3-yl)-5-phenyl-1H-pyrazole-1-carboxamide (**9k**): Pale yellow solid; yield 83%; mp 218-220°C; ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6): δ 3.54 (s, 3H, OCH_3), 6.81-7.79 (m, 15H, Ar—H), 8.86 (s, 1H, HC═NCO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 59.6 (OCH_3), 1101, 112.4, 119.6, 121.4, 122.7, 123.5, 124.9, 126.2, 127.4, 128.6, 129.6, 131.0, 132.7, 134.2, 143.3, 145.2, 147.4, 148.9, 150.3 (aromatic carbons), 162.6 (HC̲═NH), 171.4 (C̲O—O), 180.5 (N—C̲O—N) ppm; HRMS: *m/z* Calcd. for $C_{27}H_{20}N_3O_4$ $(M + H)^+$ 450.1454; Found 450.1450.

*(E)-N-(2-Chlorobenzylidene)-3-(1-oxo-1H-isochromen-3-yl)-5-phenyl-1H-pyrazole-1-carboxamide (**9l**)*: Light yellow solid; yield 68%; mp 214-216°C; ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6): δ 6.76-7.84 (m, 15H, Ar—H), 8.93 (s, 1H, HC═NCO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 109.8, 113.0, 117.4, 120.3, 123.2, 123.9, 125.8, 126.9, 127.5, 128.3, 129.1, 130.4, 131.5, 132.3, 134.2, 135.1, 141.6, 144.0, 146.2, 149.2, 150.7 (aromatic carbons), 161.0 (HC̲═NH), 171.5 (C̲O—O), 182.3 (N—C̲O—N) ppm; HRMS: *m/z* Calcd. for $C_{26}H_{17}ClN_3O_3$ $(M + H)^+$ 454.0958; Found 454.0955.

2.1.4 | Biological experiment

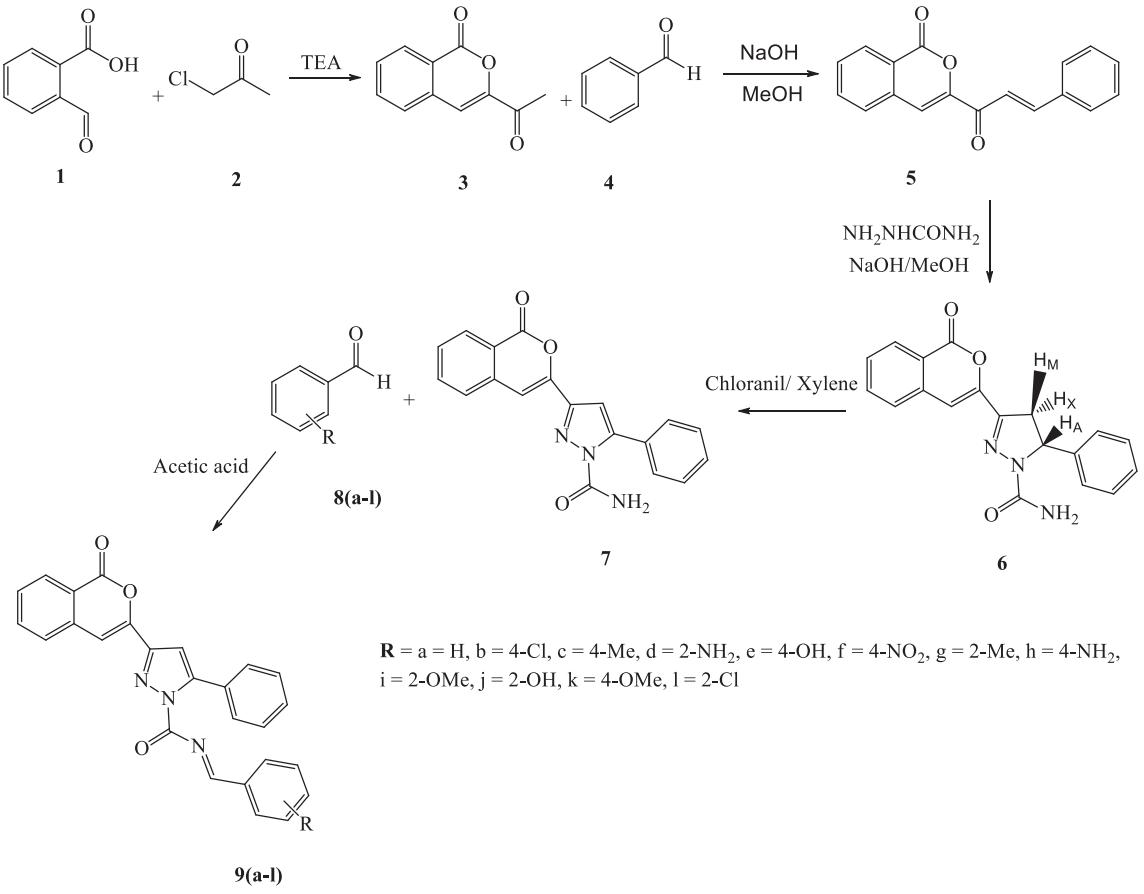
Antimicrobial activity

Four bacteria namely *Proteus vulgaris* (ATCC-29213), *Escherichia coli* (ATCC-8739), *Staphylococcus aureus* (ATCC-19433), and *Bacillus subtilis* (ATCC-6633) were used to screen the antibacterial nature of the synthesized compounds. For antifungal tests, two fungi namely *Aspergillus flavus* (MTCC-1884) and *Aspergillus niger* (MTCC-1881) were utilized. Chloramphenicol and Ketoconazole were used as reference drugs for antibacterial and antifungal tests, respectively. According to the methodology cited there in,[46] two different test concentrations 50 and 100 μg/well were used.

3 | RESULTS AND DISCUSSION

3.1 | Chemistry

The preparation method that deliver to the production of the titled compounds **6**, **7**, and **9**(**a**-**l**) were figured out in Scheme 1. By implementing the standard literature, reaction of formyl benzoic acid (**1**) with mono-chloroacetone (**2**) formed the product 3-acetyl-1*H*-isochromen-1-one (**3**).



SCHEME 1 Synthesis of tri-substituted pyrazole derivatives

TABLE 1 Compounds **6**, **7** and **9**(**a-l**) in vitro antibacterial results

Test compounds number	Test concentration (μg/well)	Inhibition zone (mm)			
		Gram-negative bacteria		Gram-positive bacteria	
		Proteus vulgaris (ATCC-29213)	*Escherichia coli* (ATCC-8739)	*Staphylococcus aureus* (ATCC-19433)	*Bacillus subtilis* (ATCC-6633)
6	50	03	06	05	07
	100	05	07	07	08
7	50	05	07	08	09
	100	07	08	10	12
9a	50	10	15	13	16
	100	12	21	16	19
9b	50	25	30	27	31
	100	30	37	34	36
9c	50	07	11	09	10
	100	09	16	13	15
9d	50	0	0	0	0
	100	0	0	0	0
9e	50	22	29	25	28
	100	26	34	31	32
9f	50	26	31	29	35
	100	32	39	35	43
9g	50	08	12	11	13
	100	10	20	14	18
9h	50	0	0	0	0
	100	0	0	0	0
9i	50	13	18	14	18
	100	16	24	19	21
9j	50	19	23	19	22
	100	21	31	25	26
9k	50	15	21	16	20
	100	19	27	20	24
9l	50	20	26	22	25
	100	25	33	28	30
Chloramphenicol	50	30	35	32	41
	100	36	40	38	43
Control (DMSO)		—	—	—	—

Reaction between compound **3** and benzaldehyde in presence of base such as sodium hydroxide got intermediate product **5**. 3-(1-oxo-1*H*-isochromen-3-yl)-5-phenyl-4,-5-dihydro-1*H*-pyrazole-1-carboxamide (**6**) was synthesized by using 3-cinnamoyl-1*H*-isochromen-1-one (**5**) intermediate and hydrazine carboxamide in methanol solvent media which contained sodium hydroxide base. The proton NMR spectra of compound **6** indicated an AMX intense pattern for the hydrogens of pyrazoline structure. There were three double doublet signals displayed at δ 3.20, 3.86 and 5.36 ppm were recognized to H_A, H_M, and H_X. Dehydrogenation of compound **6** with chloranil in the presence of xylene as a solvent gave the outcome of 3-(1-oxo-1H-isochromen-3-yl)-5-phenyl-1H-pyrazole-1-carboxamide (**7**) as a product. The absence of AMX splitting pattern indicated that formation of the product **7**. Further, condensation reaction was done between compound **7** and substituted aldehydes **8**(**a-l**) in

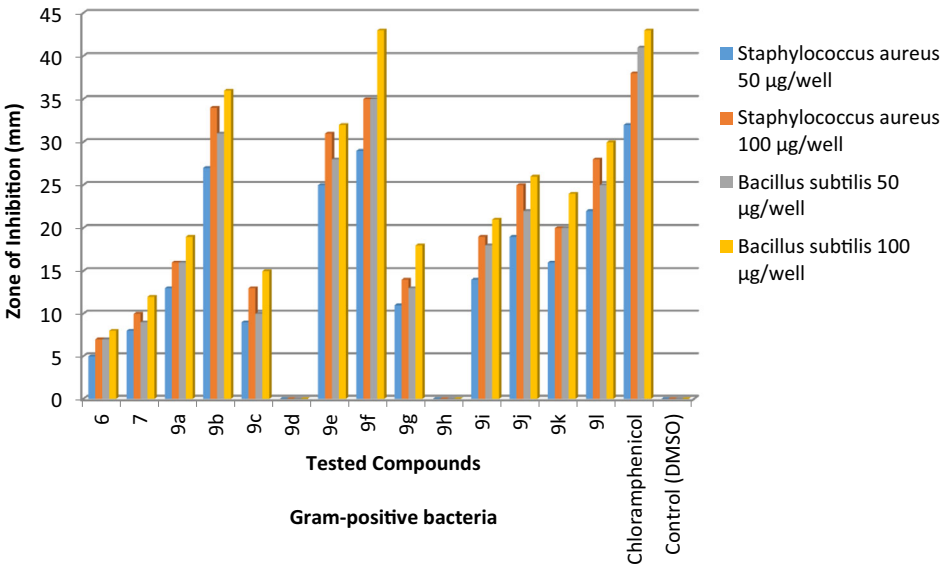


FIGURE 1 Antibacterial activity of synthesized compounds **6**, **7**, and **9** (**a**-**l**) toward Gram-positive bacteria [Colour figure can be viewed at wileyonlinelibrary.com]

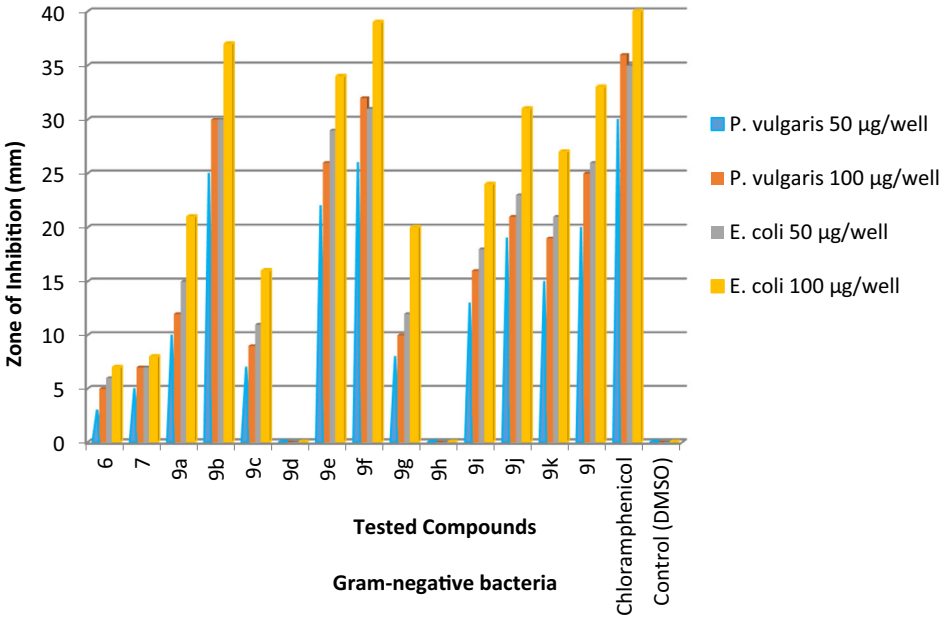


FIGURE 2 Antibacterial activity of synthesized compounds **6**, **7**, and **9** (**a**-**l**) toward Gram-negative bacteria [Colour figure can be viewed at wileyonlinelibrary.com]

acetic acid media resulted the corresponding final compounds **9**(**a**-**l**). All the final compounds formed with good yield. In total, 14 new synthetic compounds were characterized by using proton NMR, carbon NMR and HRMS mass spectral data.

3.2 | Antimicrobial activity

Antibacterial activity results tabulated in Table 1 exposed that most of the tested compounds displayed excellent to moderate activity except two compounds. The structures **9d** and **9h** displayed zero activity toward all the Gram-negative bacteria (GNB) and GPB. Between the active substances, compound **9f** displayed promising antibacterial activity towards all the bacteria in two concentrations (Figures 1 and 2). In addition, compared to reference drug Chloramphenicol, this compound showed equal effectiveness toward *B. subtilis* at 100 µg/well concentration (Figure 1). Moreover, compounds **9b**, **9e**, **9l**, and **9j** were revealed comparable antibacterial activity, when compared to the reference standard drug. In fact, antibacterial activity of aromatized and non-aromatized compounds was interested that, aromatized compound **7** have better activity than the non-aromatized compound **6**. On the other hand, Table 2 exposed the antifungal results of the test compounds **6**, **7** and **9**(**a**-**l**). In those, the dehydrogenated (**7**) and non-aromatized (**6**) compounds inactive towards to fungi, the other screened compounds demonstrated admirable to low antifungal activity.

TABLE 2 Compounds **6**, **7** and **9** (**a**-l) in vitro antifungal results

Test compounds number	Test concentration (μg/well)	Inhibition zone (mm)	
		Aspergillus flavus (MTCC-1884)	*Aspergillus niger* (MTCC-1881)
6	50	0	0
	100	0	0
7	50	06	09
	100	10	13
9a	50	14	16
	100	17	21
9b	50	33	37
	100	35	40
9c	50	09	10
	100	10	15
9d	50	0	0
	100	0	0
9e	50	31	33
	100	30	35
9f	50	36	40
	100	39	44
9g	50	10	12
	100	13	18
9h	50	0	0
	100	0	0
9i	50	16	20
	100	19	25
9j	50	24	28
	100	27	30
9k	50	20	23
	100	24	27
9l	50	28	31
	100	29	33
Ketoconazole	50	38	42
	100	41	46
Control (DMSO)		—	—

3.3 | MIC, MBC/MFC test results

From the above all biologically active compounds, three compounds were chosen to evaluate their MIC (minimum inhibitory concentration) and minimum bactericidal/fungicidal concentration (MBC/MFC) efficiency toward two fungi and four bacteria. Results in Table 3 revealed that prepared compound **9f** minimum bactericidal concentration is 2× MIC in case of two GPB, while the same compound MFC is 2× MIC toward *A. niger* fungi. In addition, MBC value of **9b** is 2× MIC toward *B. subtilis* only. Meanwhile, the other compound **9e** exhibited MBC/MFC results three or more times higher than their MIC value.

3.4 | Thought-provoking points about the structure activity relationships

Based on the biological activity results, some exciting points were observed accurately. Firstly, in between compounds **6** and **7**, there was a strong variation about their antimicrobial activities. The antibacterial activity of motif **6** is almost negligible and inactive toward fungi. This may be due to the

TABLE 3 Compounds **9f**, **9b**, and **9e** MIC (MBC/MFC) results

Samples	MIC (MBC/MFC)					
	Proteus vulgaris	***Escherichia coli***	***Staphylococcus aureus***	***Bacillus subtilis***	***Aspergillus flavus***	***Aspergillus niger***
9b	100 (>200)	50 (>100)	50 (>100)	25 (50)	50 (>100)	25 (100)
9e	50 (>100)	50 (200)	25 (200)	25 (>100)	25 (100)	50 (>200)
9f	50 (>100)	25 (100)	25 (50)	12.5 (25)	50 (>100)	50 (100)
Ciprofloxacin	12.5	12.5	6.25	6.25	—	—
Ketoconazole	—	—	—	—	12.5	6.25

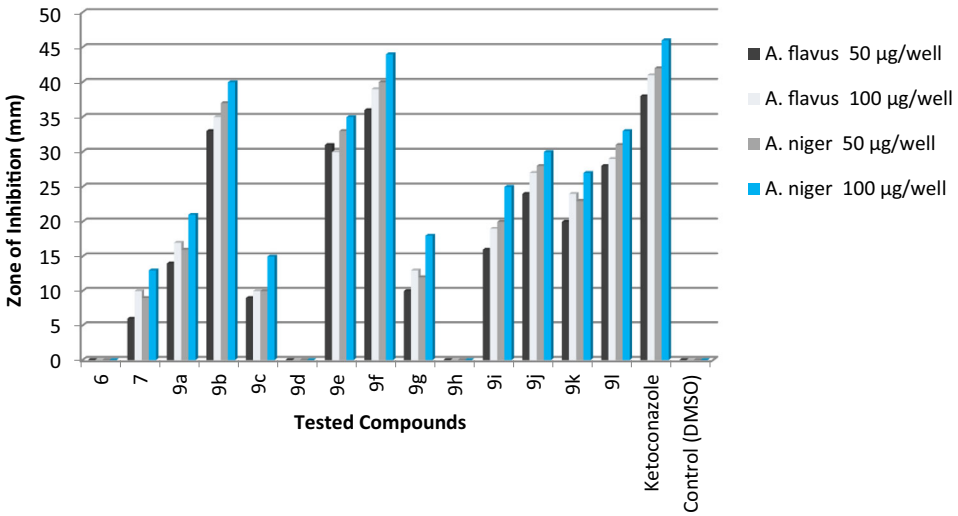


FIGURE 3 Antifungal activity of synthesized compounds **6**, **7**, and **9**(**a**-**l**) [Colour figure can be viewed at wileyonlinelibrary.com]

presence non-aromatized pyrazole core. While the composite **7** displayed antibacterial and antifungal nature, because of its aromatized pyrazole structure. The most valid point observed was that compounds **6** and **7** exhibited less antimicrobial activity than amalgams **9**(**a**-**l**). The strong variation was happened in biological results only due to the presence of the extended conjugation presented in compounds **9**(**a**-**l**). Later, about the tested compounds **9**(**a**-**l**) and their activity results delivered some interesting key points. Between the compounds **9**(**a**-**l**), all active compounds displayed excellent antibacterial activity toward GPB particularly, *B. subtilis* than GNB. In fact, total active compounds showed higher antifungal activity toward *A. niger* in two concentrations than other fungi (Figure 3). On the other hand, may be due to the presence of the amino group in the compounds **9d** and **9h** that have zero antimicrobial activity. Similarly, compound **9f** showed higher antimicrobial activity when compared to all other active compounds. This may be due to the presence of a nitro group as an attachment.

4 | CONCLUSION

A series of novel tri-substituted pyrazole derivatives were prepared via reactive intermediates and tested for their antibacterial and antifungal activities. Between two intermediates **6** and **7**, aromatized intermediate compound **7** showed better antimicrobial action than compound **6**. Among the condensation products **9**(**a**-**l**), expect two, remaining compounds displayed excellent to low antimicrobial assay. Interestingly, all the active compounds strongly effective toward GPB mainly, *B. subtilis*, while the total active compounds delivered higher antifungal action toward *A. niger* fungi than other fungi. The nitro-substituted compound **9f** exposed higher biological activity. On the other hand, amino attached tri-substituted pyrazole compounds do not show any activity against tested all microbial strains.

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