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Novel chalcones and 1,3,5-triphenyl-2-pyrazoline derivatives as antibacterial agents

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The authors Ponnurengam Malliappan Sivakumar, Suresh Ganesan and Prabhawathi Veluchamy wishes to dedicate this work to their guide Prof MD on the occassion of his birthday

Novel sixteen chalcones and thirteen 1,3,5-triphenyl-2-pyrazolines were synthesized and characterized using FT-IR, HR-Mass, NMR (^{1}H-NMR, ^{13}C-NMR, 135 DEPT, ^{1}H–^{1}H CoSY and ^{1}H and ^{13}C CoSY) and XRD. These compounds were evaluated for their antibacterial activity against six micro-organisms, namely *Bacillus subtilis* NCIM 2718, *Staphylococcus aureus* NCIM 5021, *Salmonella typhi* NCIM 2501, *Enterobacter aerogenes* NCIM 5139, *Pseudomonas aeruginosa* NCIM 5029, and *Proteus vulgaris* NCIM 2813 by twofold dilution method using resazurin as the indicator dye. In the case of chalcones, compounds with hydroxyl and bromo substitutions in the B-ring favor activity and benzyloxy substitution irrespective of its position in the A-ring. In the case of 1,3,5-triphenyl-2-pyrazolines, chloro substitution in the A-ring favors activity. Hydrophilic/lipophilic balance of the compounds plays a major role in their antibacterial activity.

Key words: 1,3,5-triphenyl-2-pyrazolines, antibacterial, resazurin

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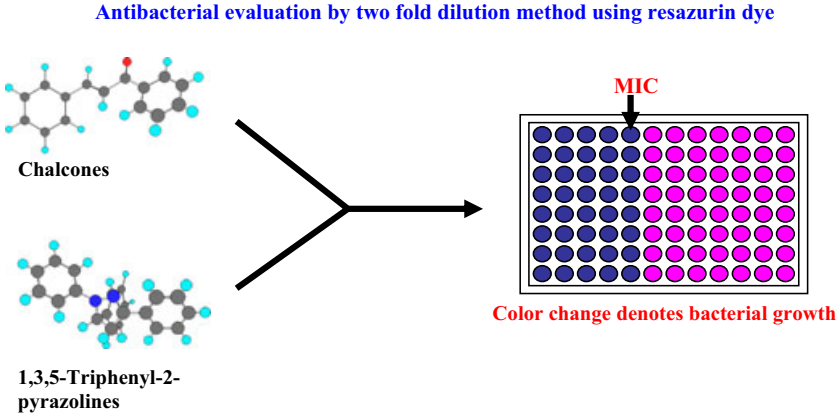
Bacterial infection and their emergence to resistance toward antibiotics is a serious threat to present and future populations. These resistant bacteria are involved in diseases including diarrhea, respiratory tract infections, meningitis, sexually transmitted infections, and hospital-acquired infections (nosocomial infections).[a] The use of more antibiotics or less antibiotics leads to the development of drug resistance in bacterial strains. These resistant strains cause fatal morbidity and also death in patients who are already infected with the disease and hence are immunocompromised (1). Clinical isolates of *Escherichia coli* O157 from human, cattle, swine, and food sources were found to be resistant against the frontline antibiotics, tetracycline, sulfa drugs, cephalosporins, and penicillins (2). β-lactamases produced by *E. coli* has developed resistance to β-lactam antibiotics (3). It is found that the mutations that affect the expression of chromosomal class I, β-lactamase expression in *Pseudomonas aeruginosa* and *Proteus vulgaris* can lead to resistance against many newer β-lactam antibiotics (4). Seventy to eighty percentage of *Staphylococcus aureus* strains are resistant against penicillins, whereas methicillin was effective until 1980. Methicillin-resistant *S. aureus* produces a penicillin-binding protein PBP2a, which binds less with methicillin and other β-lactams that are present in the bacterial cell wall (3). Vancomycin was effective against methicillin-resistant *S. aureus*, but resistance against glycopeptide class of vancomycin had delineated the therapy (5). *Salmonella typhi* responds to ciprofloxacin by inducing double mutation in *gyrA* and single mutation in *parC* genes (6). Streptomycin-resistant *Bacillus subtilis* has two mutations namely *fun* and *strR* (7).



Although methicillin, vancomycin and linezolid are designed to combat the problem of antibiotic resistance, the micro-organisms have developed resistance to these drugs as well. So there is a need for newer drugs to treat the bacterial infections and combat the bacterial resistance. Hence, medicinal chemists have to discover new chemical moieties that may exhibit anti-infective properties. Chalcones are such small molecules that have been known to exhibit a wide range of activities including antibacterial, antifungal, antitubercular, and antioxidant. Pyrazolines are another class of small molecules that can be prepared using chalcones as the starting material.

Alcaraz *et al.* (8) tested eleven natural and synthesized chalcones for antistaphylococcal activity against methicillin-resistant *S. aureus* and showed the importance of the carbonylic region for its performance. Asebogenin, which was isolated from licorice, was active against methicillin-resistant *S. aureus* (MRSA) with an IC_{50} of 4.5 μg/mL (9). Earlier, our research has proved that chalcones are very active against Gram-positive and Gram-negative bacteria (10), fungi (11), and resistant antimycobacterial strain, *Mycobacterium tuberculosis* $H_{37}Rv$ (12).

1-Aryloxy-3-aryl-5-hydroxy-5-aryl-pyrazolines (13), sulfone-linked bis heterocycle pyrazolines in combination with thiadiazoles, oxadiazoles, and triazoles (14), N1-substituted 3,5-diphenyl pyrazolines (15) have been evaluated as antibacterial agents by other researchers.

Hydrophobic substitutions in the A-ring of chalcone are considered to be more active (16). Hence, we attempted to synthesize chalcones A-ring with benzyloxy and chloro substitutions at R_1 and R_2 positions (in the A-ring) and their corresponding 1,3,5-triphenyl-2-pyrazolines (substitutions at R_1) to test their antibacterial activity. These compounds are novel and their synthesis has not been reported elsewhere.

Materials and Methods

All the chemicals used for the synthesis and antibacterial evaluations were purchased from Sigma-Aldrich (St. Louis, MO, USA), Hi-media (Mumbai, India) and SRL (Mumbai, India) and the bacterial strains (*B. subtilis* NCIM 2718, *S. aureus* NCIM 5021, *S. typhi* NCIM 2501, *Enterobacter aerogenes* NCIM 5139, *P. aeruginosa* NCIM 5029, and *Proteus vulgaris* NCIM 2813) were purchased from National Chemical Laboratory, Pune, India. The following scheme shows the synthesis of chalcones and 1,3,5-triphenyl-2-pyrazolines:

Synthesis of chalcones

The various substituted chalcones were synthesized based on the method described by Lin *et al.* (17). Ten millimoles of the corresponding substituted benzaldehydes and 1 mmol of the acetophenone derivatives were added together with methanol (10 mL) and stirred at room temperature. Two molar NaOH solutions was added to the above mixture and maintained under rapid stirring at room temperature. In majority of the cases, an almost pale yellowish solid precipitate was obtained, which was washed with 50% ethanol followed by water, recrystallized and then dried. The yield in most of the cases was more than 80%.

Synthesis of 1,3,5-triphenyl-2-pyrazolines

Appropriate chalcone (4 mmol) was mixed with 8 mmol of phenylhydrazine and 10 mL of acetic acid (17). It was boiled under reflux for 2–7 h. The synthesized 1,3,5-triphenyl-2-pyrazoline usually precipitated out on cooling, but in some cases, it was precipitated out by adding 50% of aqueous ethanol. In majority of the cases, the yield was more than 70%.

Antibacterial activity evaluation

In vitro antibacterial inhibitory activities (MIC) of the chalcones and 1,3,5-triphenyl-2-pyrazolines were determined by microdilution broth assay method (18) using resazurin as an indicator (19). Muller-Hinton broth was used to culture the bacterial strains to a final inoculum size of 5×10^5 CFU/mL. The chalcones and 1,3,5-triphenyl-2-pyrazolines were dissolved in absolute ethanol to a concentration of 10 mg/mL (20). Serially diluted chalcone and 1,3,5-triphenyl-2-pyrazoline solutions were added to successive wells in a 96-well microtitre plate and incubated with respective micro-organism for 18 h at 37 °C. After the incubation period, 10 μL of 0.01% resazurin solution was added and incubated for 2 h. The color change was assessed visually. Growth of organism changed the color from blue to pink. Growth and sterility controls were also maintained during the experiment. Test compounds serially diluted with ethanol were also kept in the 96-well microtitre plates (uninoculated dilution) to determine whether they precipitated out during the course of the experiments. One entire column had antibiotics as a positive control (norfloxacin/erythromycin). A blank assay with ethanol alone was taken into account to discount any possible effect of the solvent.

Results and Discussions

The chalcones and 1,3,5-triphenyl-2-pyrazolines were synthesized (Tables 1 and 2) and characterized by NMR (^{1}H, ^{13}C, DEPT, ^{1}H–^{1}H CoSY, ^{1}H–^{13}C CoSY), mass (HR-MS) spectroscopy. Reference (**21**) gives the NMR, HR-MS data for a representative chalcone and 1,3,5-triphenyl-2-pyrazoline. The synthesized compounds were tested for antibacterial activity against six bacterial strains including Gram-positive and Gram-negative, namely *S. aureus* NCIM 5021, *B. subtilis* NCIM 2718, *P. vulgaris* NCIM 2813, *E. coli* NCIM 2931, *S. typhi* NCIM 2501, and *P. aeruginosa* NCIM 5029 by twofold dilution assay using reaszurin as an indicator. Tables 1 and 2 lists the antibacterial

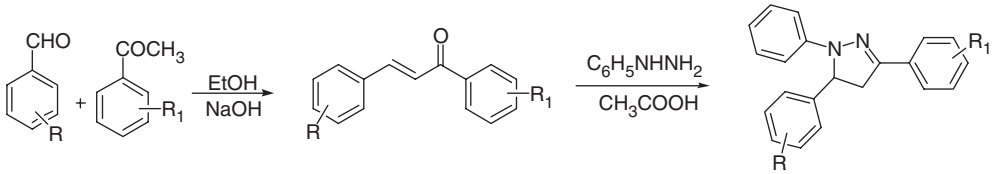
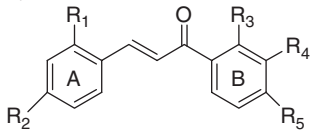
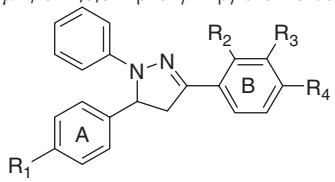


Table 1: Structure and antibacterial activities (in μM) of chalcones



	Substitutions at A-ring		Substitutions at B-ring			Antibacterial activity (against Gram-positive organisms)		Antibacterial activity (against Gram-negative organisms)			
Compound No	R_1	R_2	R_3	R_4	R_5	*Bacillus subtilis*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Salmonella typhi*	*Proteus vulgaris*
PMS-1		$C_6H_5CH_2O$			OCH_2CH_3	0.349	0.697	0.349	0.174	0.174	0.174
PMS-2		$C_6H_5CH_2O$			F	0.376	0.376	0.376	0.188	0.188	0.188
PMS-3		$C_6H_5CH_2O$		Br		0.636	0.636	0.318	0.020	0.159	0.159
PMS-4		$C_6H_5CH_2O$			OH	0.378	0.378	0.378	0.012	0.189	0.095
PMS-5		$C_6H_5CH_2O$	OH			0.378	0.378	0.189	0.024	0.189	0.095
PMS-6		$C_6H_5CH_2O$	Cl			0.358	0.717	0.358	0.179	0.179	0.179
PMS-7		Cl			OCH_2CH_3	0.436	0.872	0.436	0.218	0.218	0.218
PMS-8		Cl	OCH_3		OCH_3	0.413	0.826	0.413	0.206	0.206	0.103
PMS-9		Cl			F	0.479	0.479	0.479	0.240	0.240	0.240
PMS-10		Cl	Cl			0.451	0.902	0.451	0.226	0.113	0.226
PMS-11		Cl		Br		0.777	0.777	0.389	0.194	0.194	0.194
PMS-12		Cl		OCH_2O		0.872	0.872	0.436	0.218	0.218	0.218
PMS-13		Cl		OCH_3	OCH_3	0.826	0.826	0.413	0.206	0.206	0.206
PMS-14		Cl		OCH_3		0.458	0.917	0.458	0.229	0.115	0.229
PMS-15		Cl			Cl	0.902	0.902	0.451	0.226	0.226	0.226
PMS-16	$C_6H_5CH_2O$			Br		0.636	0.318	0.318	0.159	0.318	0.159
PMS-17	$C_6H_5CH_2O$				F	0.376	0.376	0.376	0.188	0.376	0.188
Ampicillin						0.168	0.084	0.084	0.005	0.084	0.168
Tetracycline						0.001	0.002	0.002	0.001	0.004	0.001

Table 2: Structure and antibacterial activities (in μM) of 1,3,5-triphenyl-2-pyrazoline derivatives



	Substitutions at A-ring	Substitutions at B-ring			Antibacterial activity (against Gram-positive organisms)		Antibacterial activity (against Gram-negative organisms)			
Compound No	R_1	R_2	R_3	R_4	*Bacillus subtilis*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Salmonella typhi*	*Proteus vulgaris*
PMSPY-1	$C_6H_5CH_2O$			OCH_2CH_3	1.115	0.557	0.557	1.115	0.139	0.139
PMSPY-2	$C_6H_5CH_2O$			F	2.367	2.367	1.183	0.592	0.148	0.148
PMSPY-3	$C_6H_5CH_2O$		Br		1.034	1.034	0.517	1.034	0.259	0.129
PMSPY-4	$C_6H_5CH_2O$	OH			0.595	0.297	0.297	1.189	0.297	0.149
PMSPY-5	Cl			OCH_2CH_3	0.663	0.332	0.332	2.653	0.332	0.166
PMSPY-6	Cl	OCH_3		OCH_3	0.636	0.318	0.159	0.636	0.159	0.159
PMSPY-7	Cl			F	0.713	0.356	0.356	0.713	0.178	0.178
PMSPY-8	Cl	Cl			0.681	0.340	0.170	0.681	0.170	0.170
PMSPY-9	Cl		Br		0.304	0.304	0.152	0.607	0.152	0.076
PMSPY-10	Cl		$-OCH_2O-$		0.663	0.332	0.332	0.663	0.166	0.166
PMSPY-11	Cl		OCH_3	OCH_3	0.636	0.318	0.159	0.636	0.159	0.159
PMSPY-12	Cl		OCH_3		0.689	0.689	0.172	0.344	0.172	0.172
PMSPY-13	Cl			Cl	0.681	0.681	0.170	0.681	0.170	0.170
Ampicillin					0.168	0.084	0.084	0.005	0.084	0.168
Tetracycline					0.001	0.002	0.002	0.001	0.004	0.001

activity of chalcones and 1,3,5-triphenyl-2-pyrazolines, respectively, in terms of micromolar (μM) concentration.

Chalcones **PMS-1, PMS-3, PMS-4, PMS-5**, and **PMS-16** were more active (at least against four of the six strains) and compounds **PMS-9, PMS-10, PMS-14**, and **PMS-15** were least active (at least against four of the six strains) in general when compared to others against all six bacterial strains.

The most active compounds have benzyloxy substitution either in the R_1 or in the R_2 position in A-ring which is the hydrophobic ring (10) in chalcone, and compounds **PMS-3** and **PMS-16** have bromine in their R_4 position of the B-ring. It is also observed that compounds which have substitution at R_4 when compared to R_5 and R_3 positions were more active, which was observed by other researchers too (16,22). It is found that the substitution in the B-ring is important for antibacterial activity (22). Compounds **PMS-4** and **PMS-5** have hydroxyl substitution in their B-ring at R_5 and R_3 position. Our earlier research and other researchers too have found the importance of hydroxyl and bromo substitutions in B-ring for enhanced antibacterial activity (10,16,23,24). Compound **PMS-1** has ethoxy substitution in R_5 position of the B-ring. Alkoxy substitution in R_5 position of the B-ring was also found to be important for the antibacterial activity (16). The least active compounds have chloro substitution in their A-ring. The chloro-substituted chalcones (in A-ring) also exhibit activity when they have polar substitutions such as hydroxyl in their R_4 position of the B-ring (25). Compounds **PMS-9, PMS-10**, and **PMS-15** have halogen substitution in their B-ring. Lipophilic/hydrophilic balance appears to determine the antibacterial activity of chalcones. Even though increase in the hydrophobic surface increases the antibacterial activity, it appears that the dipole and quadropole moments related to polarizability of the molecules are also important (26).

In the case of 1,3,5-triphenyl-2-pyrazolines, compounds **PMSPY-6, PMSPY-9, PMSPY-10**, and **PMSPY-11** were more active against at least four micro-organisms. The most active compounds have chloro substitution in R_1 position at A-ring. Other researches also found that chloro substitution at R_1 position in quinoline-based 2-pyrazolines exhibits very good activity when compared to chloramphenicol against *S. aureus, S. typhi*, and *E. coli* (27). Among the most active compounds, **PMSPY-6** and **PMSPY-11** have dimethoxy substitution in B-ring. Compound **PMSPY-9** has bromine substitution at R_3 position in the B-ring. Even though compound **PMSPY-3** has bromine substitution in B-ring, it is inactive and the reason remains unclear. Compounds **PMSPY-1, PMSPY-2**, and **PMSPY-3** can be classified as least active, and interestingly these compounds have benzyloxy substitution in the A-ring.

Conclusion

In conclusion, the chalcones and 1,3,5-triphenyl-2-pyrazolines were synthesized and characterized. The synthesized compounds were also tested for antibacterial activity against six bacterial strains. In the case of chalcones, compounds **PMS-1, PMS-3, PMS-4, PMS-5**, and **PMS-16** were found to be more active and compounds **PMS-9, PMS-10, PMS-14**, and **PMS-15** were least active. The more active compounds have essential features like hydroxyl substitution in B-ring, substitution at R_4, and hydrophobic substitution like benzyloxy in the A-ring. Chloro substitution in A-ring leads to medium to lower active compounds. In the case of 1,3,5-triphenyl-2-pyrazolines, compounds **PMSPY-6, PMSPY-9, PMSPY-10**, and **PMSPY-11** were the most active ones and compounds **PMSPY-1, PMSPY-2**, and **PMSPY-3** were the least active. All the most active 1,3,5-triphenyl-2-pyrazolines have chloro substitution in their A-ring at R_1 position. The least active compounds **PMSPY-1, PMSPY-2**, and **PMSPY-3** have benzyloxy substitution in their A-ring at R_1 position. In conclusion, lipophilic/hydrophilic balance is required for antibacterial activities of chalcones and 1,3,5-triphenyl-2-pyrazolines. Our studies revealed that, in the case of chalcone, A-ring requires slightly hydrophobic substitution including benzyloxy substitution and B-ring requires hydrophilic substitution including hydroxyl and bromo groups. It is opposite in the case of 1,3,5-triphenyl-2-pyrazolines. Chloro substitution is required in A-ring for the activity and slightly hydrophilic substitution like dimethoxy and methylene dioxy compared to hydroxyl substitution that is required for good antibacterial activity. A detailed group-based QSAR (GQSAR) could give more information on structure–activity relationship, which will be performed and published later. This research gives additional knowledge to researchers to design newer chalcones and 1,3,5-triphenyl-2-pyrazolines exhibiting antibacterial activity.

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21. Spectral data for compound **PMS -10: FTIR (KBr) 1665 cm^{-1}(C=O).** ^{1}H NMR (500 MHz): δ 7.12 (1H, d, J = 16 Hz), δ 7.34–7.37 (3H, m), δ 7.40 (1H, s), δ 7.42 (1H, d, J = 1.5 Hz), δ 7.43–7.44 (2H, m), δ 7.46–7.49 (3H, m). ^{13}C NMR (500 MHz): δ193.41, 144.48, 138.95, 136.80, 132.94, 131.57, 131.31, 130.33, 129.71, 129.68, 129.40, 129.37, 129.29, 126.92, 126.63. HR-MS (m/z) for molecular formula $C_{15}H_{10}Cl_2O$: calculated = 277.0631, found = 277.0631. Spectral data for compound **PMS – 13: FTIR (KBr) 1665 cm^{-1}(C=O).** ^{1}H NMR (500 MHz): δ 3.97 (6H, s), 6.93 (1H, d, J = 8 Hz), δ 7.37 (2H, dd, J = 8.5 Hz), δ 7.56–7.58 (2H, m), δ 7.61 (1H, d, J = 2 Hz), δ 7.67 (1H, dd, J = 2 Hz, J' = 8.5 Hz), δ 7.75 (1H, d, J = 15.5 Hz). ^{13}C NMR (500 MHz): δ 56.05, 56.10, 109.98, 110.76, 122.08, 123.05, 129.16,129.19, 129.22, 129.50, 131.14, 133.57, 136.18, 142.43, 149.31, 153.41, 188.23. HR-MS (m/z) for molecular formula $C_{17}H_{15}ClO_3$: calculated = 303.0784, found = 303.0788. Spectral data for compound **PMSPY – 7: FTIR (KBr) 1587 cm^{-1}(C=N).** ^{1}H NMR (500 MHz): δ 3.06 (1H, dd, J = 7.5 Hz, J' = 17 Hz), δ 3.80 (1H, dd, J = 12.5 Hz, J' = 17 Hz), δ 5.23 (1H, dd, J = 7.5 Hz, J' = 12.5 Hz), δ 6.78–6.81 (1H, m), δ 7.01–7.03 (2H, m), δ 7.05–7.08 (2H, m), δ 7.16–7.19 (2H, m), δ 7.23–7.25 (2H, m), δ 7.29–7.31 (2H, m), δ 7.68–7.69 (2H, m). ^{13}C NMR (500 MHz): δ 43.55, 64.00, 113.38, 115.57, 115.75, 119.44, 127.31, 127.51, 127.48, 127.54, 128.82, 128.84,128.99, 129.02, 129.37, 133.39, 140.94, 144.63, 145.80, 162.08, 164.06. HR-MS (m/z) for molecular formula $C_{21}H_{16}ClFN_2$: calculated = 351.1064, found = 351.1064. Spectral data for compound **PMSPY – 11: FTIR (KBr) 1587 cm^{-1}(C=N).** ^{1}H NMR (500 MHz): δ 3.05 (1H, dd, J = 7.5 Hz, J' = 17 Hz), δ 3.78 (1H, dd, J = 12.5 Hz, J' = 17 Hz), δ 3.89 (3H, s), δ 3.97 (3H, s), δ 5.18 (1H, dd, J = 7.5 Hz, J' = 12.5 Hz), δ 6.77–6.83 (2H, m), δ 7.00–7.03 (3H, m), δ 7.16–7.19 (2H, m), δ 7.23–7.30 (4H, m), δ 7.48 (1H, d, J = 1.5 Hz). ^{13}C NMR (500 MHz): δ 43.65, 55.95, 63.92, 108.14, 110.67, 113.33, 119.12, 119.19, 125.41, 125.28, 127.36, 128.79, 128.96, 129.09, 129.32, 129.43, 129.96, 133.28, 141.20, 144.89, 146.91, 149.18, 150.03. HR-MS (m/z) for molecular formula $C_{23}H_{21}ClN_2O_2$: calculated = 393.1373, found = 393.1370.
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Note

[a]http://www.who.int/mediacentre/factsheets/fs194/en/ [accessed on September 01 2010].