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Synthesis and Antimicrobial Activity of Piperine Analogues Containing 1,2,4-Triazole Ring

KOTTAKKI NAVEEN KUMAR[1], KARTEEK RAO AMPERAYANI[1], V. RAVI SANKAR UMMDI[2] and UMA DEVI PARIMI[3,*]

[1]Department of Chemistry, GITAM University, Visakhapatnam-530045, India
[2]Molecular Biology Division, MicGene Lab, Visakhapatnam-530017, India
[3]Department of Chemistry, Gayatri Vidya Parishad College for Degree and P.G. Courses (A), Visakhapatnam-530045, India

*Corresponding author: E-mail: umadevichemistry@gmail.com

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A series 1,2,4-triazole piperine analogues (**TP1-TP6**) were designed and synthesized. The structures were confirmed using ^{1}H NMR and ^{13}C NMR. Antibacterial study was done using Gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) and Gram-negative micro-organisms (*E. coli* and *Pseudomonas aeruginosa*) by disc diffusion method. Compound containing chloro substitution (**TP6**) showed the highest effect, while compound **TP1**, **TP3**, **TP4**, **TP5** showed the moderate activity.

Keywords: Piperine-triazole analogues, Antimicrobial activity.

INTRODUCTION

Black pepper (*Piper nigrum*) is one of the important medicinal plants grown around south Asia region with various biological activities [1], piperine is hydrophobic amide alkaloid present in *piper nigrum* which is responsible for the pungency of pepper [2-4]. Literature survey reveals that piperine possess various biological activities such as with potent antibacterial activity either individually [5] or in combination with mupirocin [6], antioxidant activity [7] and anti-inflammatory activity [8].

As the need increased for identification of new biological active molecules against emerging microbial infections, researchers synthesizing new active compounds from natural compounds as precursors or their derivatives or combining two active molecules into new bioactive (hybrid or conjugate) molecules [9,10]. This type of new molecules possess more potent biological activity in curing different diseases compared to their parent molecules [10]. 1,2,4-Triazole nucleus is stable when it incorporated into biologically active molecules increases their pharmacological activity. The previous studies on 1,2,4-triazole derivatives such as 1,2,4-triazoles-5-thione, 1,3,4-thiadiazole, 1,3-thiazolan-4-one possess antibacterial activity [9,10].

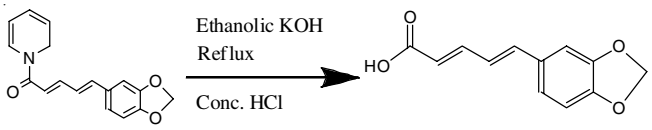
Piperine and its analogs are also one of the major compounds possess potent antibacterial activity [11,12]. The study of Kumar *et al.* [13] demonstrate that piperine analogs are inhibit the NorA pump of *Staphylococcus aureus.* There is no earlier reports on hybrid piperine analogues with 1,2,4-triazoles. In this point of view, the present study was aimed to synthesize novel piperine-1,2,4-triazoles analogues and their antibacterial activity evaluation.

EXPERIMENTAL

The solutions/reagents used in the current research were of analytical grade. The synthesized compounds confirmed through their structural elucidation using ^{1}H NMR (Bruker), ^{13}C NMR and IR their melting points (Fisher-Johns apparatus) and their retention times using thin layer chromatography.

Synthesis of piperic acid: The was prepared using 0.118 mol of piperine and 20 % EtOH-KOH (300 mL) for 10 h reflux and then washed with ethanol (99 % purity). The potassium piperate was then treated with 0.1 M HCl and then washed with distilled water (**Scheme-I**). Then piperic acid (yellow colour) was recrystallized with ethanol.

Synthesis of triazoles: Substituted benzoic acid (0.01 mol) in 0.2 mol of anhydrous methanol and 0.5 mL of conc. H_2SO_4 was added in a round bottom flask and then refluxed

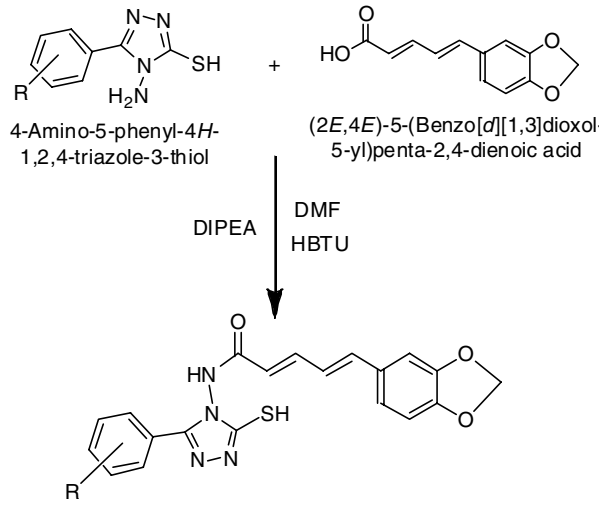


Scheme-I

for 5 h. The resultant compound was confirmed by TLC (hexane:ethyl acetate) in the ratio 80:20 and then required compound was isolated by treating with NaOH. Then 0.01 mol of substituted methyl benzoate in 25 mL of ethanol was taken in a round bottom flask. The solution was refluxed for 4 h by adding 0.7 mL of 0.15 mol N_2H_4. The product was confirmed by TLC (hexane:ethyl acetate) in the ratio 80:20 and distilledoff ethanol and it is cooled in ice water. The resultant compound was recrystallized with EtOH (78 % yield).

Potassium 2-benzoylhyrazinecarbodithioate precipitate was prepared from mixture of 8.5 g of KOH (1.20 mol) in 125 mL C_2H_5OH, 1.36 g of benzoic acid hydrazide (0.08 mol) and 14.5 mL of carbon disulphide (1.52 mol) by reacting with 250 mL anhydrous ether. The prepared precipitate was filtered by washing with diethyl ether. The potassium salt was obtained from filtrate after drying. Then, the mixture of 4.44 g (0.21 mol) potassium salt (2-benzoylhydrazine carbodithioate), 2 mL of hydrazine hydrate (0.5 mol) in 80 mL water was refluxed for 3 h, then a homogenous solutions formed after release of hydrogen sulphide and the colour of solution will turn to green. Then solution was precipitated using cold water, after precipitation acidification was carried using conc. HCl. Then, solution was filtered by washing with cold water. Then, final product was recrystallized using EtOH (**Scheme-II**).

Synthesis of piperine analogs: Piperic acid (1.98 g) was dissolved in dimethyl formamide at 0-5 °C, then added 2-(1*H*-benzotriazol-1-yl)-1,1,13,3-tetramethyluronium hexafluorophosphate (HBTU) and was continuously stirred for 30 min. After the homogenous mixture formation, added 2.6 mL N,N-diisopropyl ethylamine, 1.98 g of 4-amino-5-phenyl-4*H*-1,2,4-triazole-3-thiol (0.51 mol). After addition, the temperature was raised to room temperature and mixture was stirred continuously for 5 h at room temperature. The product formation was monitored simultaneous application of TLC (6:4 ratio of hexane and ethyl acetate as mobile phase). After the crude compound formation, it was washed separately with HCl and then NaOH. Then crude extract was concentrated and used for column chromatography to isolate pure compounds using hexane and ethyl acetate mixture as mobile phase (**Scheme-III**).

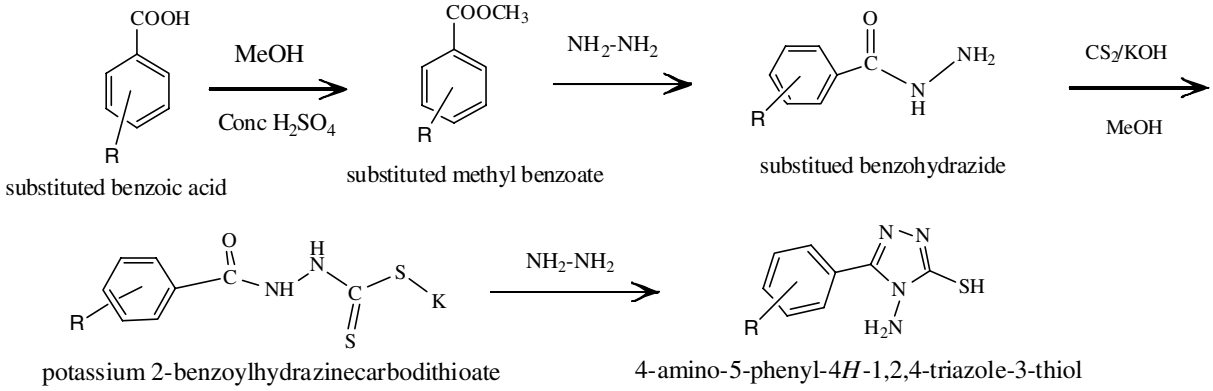


Scheme-III

Spectral data

(2*E*,4*E*)-5-(Benzo[*d*][1,3]dioxol-5-yl)-*N*-(3-mercapto-5-phenyl-4*H*-1,2,4-triazol-4-yl)penta-2,4-dienamide (TP1): m.f. $C_{20}H_{16}N_4O_3S$, m.w. 392.09, m.p. 167-172 °C. IR (KBr, ν_{max}, cm^{-1}): 943 (N-C-S str), 3085 (CH=CH str), 1690 (C=O str), 2500 (SH str), 3539 (NH str), 1550 (C=N str), 1352 (C-N str), 698 (C-S str), 1278 (NN- C str). ^{1}H NMR amide N-H δ 8.21 (s, 1H), (triazole Ar-H δ 7.45-8.02 (m, 5H), -S-H δ-4.80 (s, 1H), piperine (C=CH) δ 6.06 (d, 1H), δ -7.28 (t, 1H), δ 6.73 (t, 1H), δ -6.75 (d, 1H)), Ar-H δ 6.75-7.03 (m, 3H), O-CH_2 δ -5.96 (s, 2H), ^{13}C NMR (125 MHz, common NMR solvents) δ 167.87, 164.40, 154.26, 147.51, 147.42, 141.62, 138.85, 130.28, 129.41, 129.06, 127.30, 125.79, 124.73, 123.17, 119.59, 108.53, 106.92, 101.01.

(2*E*,4*E*)-5-(Benzo[*d*][1,3]dioxol-5-yl)-*N*-(3-(3,5-dinitrophenyl)-5-mercapto-4*H*-1,2,4-triazol-4-yl)penta-2,4-dienamide (TP2): m.f. $C_{20}H_{14}N_6O_7S$, m.w. 482.06, m.p. 260-266 °C. IR (KBr, ν_{max}, cm^{-1}): 943 (N-C-S str), 3085 (CH=CH str), 1690 (C=O str), 2500 (SH str), 3539 (NH str), 1550 (C=N



R= H, *m*-NO_2, *p*-CH_3O, *p*-OH, *p*-NO_2, *p*-Cl

Scheme-II

str), 1352 (C-N str), 698 (C-S str), 1276 (NN- C str). ^{1}H NMR amide N-H δ 8. 32 (s, 1H) triazole 3,5-nitro Ar-H δ 8.79-8.88 (m, 3H), -S-H δ-4.57 (s, 1H), piperine (C=CH) δ 6.06 (d, 1H), δ -7.28 (t, 1H), δ 6.73 (t, 1H), δ -6.75 (d, 1H)), Ar-H δ 6.75-7.03 (m, 3H), O-CH_2 δ -5.96 (s, 2H), ^{13}C NMR (125 MHz, common NMR solvents) δ 167.90, 164.40, 153.86, 147.51, 147.42, 146.87, 141.62, 138.85, 129.41, 128.01, 125.79, 124.73, 123.17, 120.33, 119.59, 108.53, 106.92, 101.01.

(2*E*,4*E*)-5-(Benzo[*d*][1,3]dioxol-5-yl)-*N*-(3-mercapto-5-(4-methoxyphenyl)-4*H*-1,2,4-triazol-4-yl)penta-2,4-dienamide (TP3): m.f. $C_{21}H_{18}N_4O_4S$, m.w. 422.1, m.p. 154-160 °C. IR (KBr, ν_{max}, cm^{-1}): 943 (N-C-S str), 3030 (CH=CH str), 1698 (C=O str), 2500 (SH str), 3530 (NH str), 1550 (C=N str), 1354 (C-N str), 698 (C-S str). ^{1}H NMR amide N-H δ 8.15 (s, 1H) triazole 4-methoxy Ar-H δ 7.09-7.79 (m, 4H), -O-CH_3 δ-3.79 (s, 1H), -S-H δ-4.80 (s, 1H), piperine (C=CH) δ 6.06 (d, 1H), δ -7.28 (t, 1H), δ 6.73 (t, 1H), δ -6.75 (d, 1H)), Ar-H δ 6.75-7.03 (m, 3H), O-CH_2 δ -5.96 (s, 2H), ^{13}C NMR (125 MHz, common NMR solvents) δ 167.87, 164.40, 160.77, 154.06, 147.51, 147.42, 141.62, 138.85, 129.41, 126.10, 125.79, 123.17, 121.15, 119.59, 114.31, 108.53, 106.92, 101.01, 55.32.

(2*E*,4*E*)-5-(Benzo[*d*][1,3]dioxol-5-yl)-*N*-(3-(4-hydroxyphenyl)-5-mercapto-4*H*-1,2,4-triazol-4-yl)penta-2,4-dienamide (TP4): m.f. $C_{20}H_{16}N_4O_4S$, m.w. 408.43, m.p. 177-182 °C. IR (KBr, ν_{max}, cm^{-1}): 943 (N-C-S str), 3109 (ArCH str), 1689 (C=O str), 2500 (SH str), 3542 (NH str), 1550 (C=N str), 1359 (C-N str), 698 (C-S str). ^{1}H NMR amide N-H δ 8.10 (s, 1H) triazole 4-hydroxy Ar-H δ 6.93-7.65 (m4H), -O-H δ-7.60 (s, 1H), -S-H δ-4.80 (s, 1H), piperine (C=CH) δ 6.06 (d, 1H), δ -7.28 (t, 1H), δ 6.73 (t, 1H), δ -6.75 (d, 1H)), Ar-H δ 6.75-7.03 (m, 3H), O-CH_2 δ -5.96 (s, 2H), ^{13}C NMR (125 MHz, common NMR solvents) δ 167.87, 164.40, 153.92, 150.65, 147.51, 147.42, 141.62, 138.85, 129.41, 126.48, 125.79, 123.17, 119.59, 119.45, 116.45, 108.53, 106.92, 101.01.

(2*E*,4*E*)-5-(Benzo[*d*][1,3]dioxol-5-yl)-*N*-(3-mercapto-5-(4-nitrophenyl)-4*H*-1,2,4-triazol-4-yl)penta-2,4-dienamide (TP5): m.f. $C_{20}H_{15}N_5O_5S$, m.w. 437.08, m.p. 235-241 °C. IR (KBr, ν_{max}, cm^{-1}): 943 (N-C-S str), 3106 (ArCHstr), 1694 (C=O str), 2500 (SH str), 3540 (NH str), 1550 (C=N str), 1345 (C-N str), 698 (C-S str). ^{1}H NMR amide N-H δ 8.33 (s, 1H) triazole 4-nitro Ar-H δ 8.01-8.30 (m, 4H), -S-H δ-4.80 (s, 1H), piperine (C=CH) δ 6.06 (d, 1H), δ -7.28 (t, 1H), δ 6.73 (t, 1H), δ -6.75 (d, 1H)), Ar-H δ 6.75-7.03 (m, 3H), O-CH_2 δ -5.96 (s, 2H), ^{13}C NMR (125 MHz, common NMR solvents) δ 167.87, 164.40, 154.26, 149.11, 147.51, 147.42, 141.62, 138.85, 132.44, 129.41, 125.79, 125.40, 124.45, 123.17, 119.59, 108.53, 106.92, 101.01.

(2*E*,4*E*)-5-(Benzo[*d*][1,3]dioxol-5-yl)-*N*-(3-(4-chlorophenyl)-5-mercapto-4*H*-1,2,4-triazol-4-yl)penta-2,4-dienamide (TP6): m.f. $C_{20}H_{15}N_4O_3SCl$, m.w. 426.06, m.p. 185-191 °C. IR (KBr, ν_{max}, cm^{-1}): 943 (N-C-S str), 3010 (Ar-CH str), 2845 (CH_2 str), 2500 (SH str), 3520 (NH str), 1550 (C=N str), 1300 (C-N str), 698 (C-S str), 698 (C-Cl str). ^{1}H NMR amide N-H δ 8.18 (s, 1H) triazole 4-chloro Ar-H δ 7.48-7.86 (m, 4H), -S-H δ-4.80 (s, 1H), piperine (C=CH) δ 6.06 (d, 1H), δ -7.28 (t, 1H), δ 6.73 (t, 1H), δ -6.75 (d, 1H)), Ar-H δ 6.75-7.03 (m, 3H), O-CH_2 δ -5.96 (s, 2H), ^{13}C NMR (125 MHz, common NMR solvents) δ 167.87, 164.40, 154.27, 147.51, 147.42, 141.62, 138.85, 137.86, 129.41, 129.22, 127.24, 126.49, 125.79, 123.17, 119.59, 108.53, 106.92, 101.01.

Antimicrobial activity

Two microorganisms *viz. Bacillus subtilis* MTCC211 and *Escherichia coli* MTCC443 are used in the present study and collected from MTCC, Chandigarh, India. Mueller Hinton Agar medium to be used for routine susceptibility testing of bacteria due to its acceptable reproducibility, satisfactory growth of most pathogens [14].

Agar-well diffusion testing: The antibacterial activity was assessed for the synthesized drugs using agar well diffusion test method [15]. The method is basically on diffusion of the desired drug in a vertical cylinder well in a agar petri plate, which is pre-cultured with the testing bacterial strain. The activity of the compounds will be measured using the formation of zones around the wells [16,17]. In the current study, Muller-Hinton agar was used to culture the test micro-organisms on petri dishes. After solidification of agar, cotton swab was used to spread the testing bacterial strains and then 6mm wells were placed on agar plate with sterile steel borer. Then, 50 µL of synthesized drugs (**TP-1** to **TP-6**) were tested on selected bacteria using vancomycin as standard drug (30 µg) and dimethl sulphoxide (DMSO) as vehicle. After, placing the test compounds, standard, vehicle in wells placed the petri dishes aside for 1 h without disturbance for diffusion of compounds in wells. Then, plates were incubated for 24 h at 37 °C. After completion of incubation, the plates were used to measure the zones of inhibition around the wells using well reader (scale). The experiment was repeated thrice and the results were expressed as average in mm. Those compounds which were unable to exhibit inhibition zone (inhibition zone diameter less than 7 mm) were considered non-active.

RESULTS AND DISCUSSION

The titled compounds were successfully synthesized with good percentage of yields and the reaction process were showed in **Scheme-III**. The synthesized compounds were confirmed by their analytical and spectral data.

The IR spectra peaks at 3300 and 1662 were corresponds to N-H of amide and C=O to the produced compound from methyl benzoate. The C-S stretch peaks at 698 cm^{-1} in IR spectra confirms of the synthesized potassium 2-benzoylhydrazine-carbodithioate compound. The IR and ^{1}H NMR spectras of 4[amino]-5-phenyl-4*H*-1,2,4-triazole-3-thiol confirms its structure by appearance of N-C-S stretch at 943 cm^{-1} and N-C-C stretch peaks at 1278 cm^{-1} IR spectra and SH, NH peaks at 4.80 δ and 8.21 δ in NMR spectra. The compound synthesized from 4[amino]-5-phenyl-4*H*-1,2,4-triazole-3-thiol is (2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)-N-(3-mercapto-5-phenyl-4*H*-1,2,4-triazol-4-yl)penta-2,4-dienamide is confirmed by its IR spectra due to absence of NH peak and presence of -N-N-C.

Antibacterial activity of synthesis compounds on bacteria: Agar well-diffusion method was used to screen the antibacterial activity of synthesized compounds (**TP-1** to **TP-6**) at 50 and 100 µg and the compounds showed concentration

dependent activity (Table-1). Among tested drugs some are more active inhibition and some are less inhibition against tested bacterial strains [*Bacillus subtilis* (Gram-positive) and *escherichia coli* (Gram-negative)]. The results of the current study describes that tested compounds were more active against Gram-positive bacteria compared to Gram-negative bacteria. **TP-2** showed highest antibacterial activity against *Bacillus subtilis*, the diameter value of zone of inhibition was 22 mm for 100 µg concentration and 15 mm for 50 µg concentration. On the other hand, standard antibiotic 30 µg vancomycine showed 32 mm zone of inhibition against the same organism. **TP-2** compounds inhibition was 31.5 % less than of the vancomycine (30 µg) rate of inhibition against the *Bacillus subtilis.* **TP-2** inhibition was followed by the **TP-6** and **TP-3** compounds. They showed 20 mm and 19 mm zone of inhibition, respectively against the *Bacillus subtilis.* Other three compounds **TP-1**, **TP-4** and **TP-5** also showed moderate inhibition activity against *Bacillus subtilis, i.e.* 12, 11 and 15 mm, respectively.

TABLE-1
MEAN VALUE OF ZONE OF INHIBITION (mm) FOLLOWED BY STANDARD ERROR OF ANTI-BACTERIAL ACTIVITY OF SELECTED COMPOUNDS

Compd.	Gram-negative (*E. coli*)		Gram-positive (*B. subtilis*)	
	100 µg	50 µg	100 µg	50 µg
TP-1	12 ± 0.21	10 ± 0.33	12 ± 0.19	10 ± 0.36
TP-2	12 ± 0.31	10 ± 0.41	22 ± 0.15	15 ± 0.45
TP-3	10 ± 0.40	8 ± 0.43	19 ± 0.45	13 ± 0.19
TP-4	10 ± 0.33	8 ± 0.15	11 ± 0.45	8 ± 0.09
TP-5	15 ± 0.45	10 ± 0.39	14 ± 0.32	9 ± 0.29
TP-6	16 ± 0.33	12 ± 0.35	20 ± 0.52	12 ± 0.17
Vancomycine (30 µg)	30 ± 1.95		32 ± 1.43	

All six compounds showed inhibition activity against Gram-negative *(Escherichia coli)* bacteria. Among them, **TP-6** showed highest antibacterial activity against *Escherichia coli*, the diameter value of zone of inhibition was 16 mm at 100 µg concentration and 12 mm at 50 µg concentration. On the other hand, standard antibiotic 30 µg vancomycine showed 30 mm zone of inhibition against the same organism. **TP-6** compounds inhibition was 46.7 % less than of the 30 µg vancomycine rate of inhibition against the *Escherichia coli.* **TP-6** rate of inhibition was followed by the **TP-5**, **TP-2** and **TP-1** compounds inhibition activity, they showed 15 mm, 12 mm and 12mm zone of inhibition, respectively against the *Escherichia coli.* Other compounds **TP-3** and **TP-4** also showed moderate inhibition activity against *Escherichia coli, i.e.* 10 mm each.

The results of the current study confirms that compounds **TP-2** and **TP-6** more active against the tested bacterial strains and was equivalent to standard drug. The structural similarity between them concludes that the existence of Nitro groups at 3rd and 5th position to benzene ring for **TP-2** and halogen group at 4th position to benzene ring exhibited excellent antibacterial activity showing importance of halogen and nitro groups in the compounds.

Conclusion

A new series of piperine-triazole analogs were synthesized with simple and efficient methods and screened for antibacterial activity and their results concludes that the presence of chloro substituted derivatives possess good antimicrobial activity against Gram-positive bacteria and 3,5-dinitrophenyl derivatives are moderately active.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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