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Original Research Article

Antimicrobial, hemolytic and thrombolytic activities of some new N-substituted-2-({5-[(1E,3E)F-4-(1,3-benzodioxol-5-yl)-1,3-butadienyl]-1,3,4-oxadiazol-2-yl}sulfanyl) propanamides

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Abstract

Purpose: *To synthesize and evaluate the bioactivity of some N-substituted-2-({5-[(1E,3E)-4-(1,3-benzodioxol-5-yl)-1,3-butadienyl]-1,3,4-oxadiazol-2-yl}sulfanyl)propanamides (9a-o) of the alkaloid piperine (1) extracted from P. nigrum (black pepper).*
Methods: *Extract 1 was subjected to basic hydrolysis to obtain piperic acid (2). The heterocyclic 1,3,4-oxadiazole ring was synthesized from 2 through the formation of an ester and carbohydrazide. A series of electrophiles, 8a-o, were synthesized in the presence of 10 % Na_2CO_3. The final compounds, 9a-o, were synthesized by stirring 5-[(1E,3E)-4-(1,3-benzodioxol-5-yl)-1,3-butadienyl]-1,3,4-oxadiazol-2-thiol (5) and 8a-o in LiH/DMF. Spectral analysis was performed using infrared (FTIR), proton nuclear magnetic resonance (1H-NMR) and electron impact mass spectrometry (EI-MS) to determine the structures of 9a-o. Antimicrobial activity was evaluated as zone of inhibition by disc diffusion method. Hemolytic and thrombolytic activities were determined by measuring absorbance before and after incubation of blood cells with test compound.*
Results: *Compound 9d strongly inhibited Bacillus subtilis and Escherichia coli with zone of inhibition values of 16 mm for each. The reference drug, rifampicin, showed zone of inhibition of 21 and 23 mm against B. subtilis and E. coli, respectively. Compound 9a strongly inhibited Aspergillus niger with a zone of inhibition of 18 mm compared to the reference drug, fluconazole, with a zone of inhibition of 19 mm.*
Conclusion: *The newly synthesized compounds are more active antimicrobial agents than piperine. Compounds 9a and 9d are the most active.*

Keywords: *Piper nigrum, Piperine, Propanamide, Hemolytic, Thrombolytic, Black pepper.*

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INTRODUCTION

Herbal drugs have a broad spectrum of therapeutic effects. *Piper nigrum* L. belongs to the family Piperaceae and has multiple uses in the food industry, perfumery and medicines [1]. 1,3,4-Oxadiazole derivatives also possess remarkable bioactivities [2-6]. The quantitative and qualitative effects of substitution on 1,3,4-ozadiazole prompted us to synthesize various propanamide derivatives with minimal toxicity and improved bioactivity. A series of propanamides bearing 1,3,4-oxadiazole and piperine moieties were synthesized with the aim of introducing new bioactive compounds.

EXPERIMENTAL

Plant material

P. nigrum L. (black pepper) was collected from Lahore, Pakistan in June 2014. Dr. Zaheerud Din, a taxonomist in the Department of Botany, Government College University, Lahore, Pakistan, authenticated the sample as *P. nigrum* L. The Herbarium of the Department of Botany, Government College University, Lahore, Pakistan retained the specimen (GC-Herb.Bot. 2970).

General

The chemical reagents and solvents employed were purchased from Sigma-Aldrich and Merck. TLC on pre-coated silica gel G-25-UV_{254} plates with ethyl acetate and *n*-hexane solvent system was performed for pre-confirmation. A Griffin and George apparatus was used to record melting points using an open capillary tube. I.R. spectra were recorded on a Jasco-320-A spectrophotometer by the KBr pellet method. The proton-NMR spectra were recorded on Bruker spectrometers operating at 300/400 MHz. The mass spectra (EIMS) were recorded on a JMS-HX-110.

Extraction of piperine alkaloid (1)

P. nigrum (1000 g) was ground and placed in a Soxhlet apparatus [7]. Methanol (1500 mL) was added, and the mixture was refluxed for 3 h. After evaporating the excess solvent, excess distilled water (250 mL) was added and the pH was adjusted to 9 - 10 with 4 % aqueous KOH. Piperine was precipitated and collected by filtration [8].

Synthesis of piperic acid (2)

Piperine (**1**; 0.05 mol) was dissolved in 100 mL of ethanol in a 250-mL round-bottom flask. Solid KOH (0.05 mol) was added and the mixture refluxed for 24 h [9]. The reaction was monitored by TLC. The pH was adjusted to 5 - 6 with dilute HCl. The precipitated piperic acid (**2**) was filtered out, washed and dried.

Synthesis of ethyl piperate (3)

Piperic acid (**2**; 5 g) was added to 150 mL of ethanol along with 2.5 mL of concentrated H_2SO_4 in a 250-mL round-bottom flask and the mixture refluxed for 7 h. Column chromatography was used to separate **3** from the reaction medium [10].

Synthesis of piperic carbohydrazide (4)

Ethyl piperate (**3**; 0.05 mol) was added to 80 mL ethanol containing hydrated hydrazine (3.0 mL) in a 250-mL round-bottom flask and the mixture refluxed for 4 h [11]. The precipitate was obtained after evaporating the excess solvent. The precipitated piperic carbohydrazide (**4**) was filtered out, washed with excess distilled water and dried.

Synthesis of 5-[(*1E,3E*)-4-(1,3-benzodioxol-5-yl)-1,3-butadienyl]-1,3,4-oxadiazol-2-thiol (5)

Piperic carbohydrazide (**4**; 0.04 mol) was mixed in 150 mL of ethanol with solid KOH (0.04 mol) in a 250-mL round-bottom flask and the mixture refluxed. Carbon disulfide (0.08 mol) was added dropwise and the mixture refluxed for 6 h. Excess distilled water (100 mL) was added and the pH was adjusted to 5 - 6 with dilute HCl [12]. The title compound was collected by filtration and dried.

Synthesis of 2-bromo-*N*-(alkyl/aralkyl/aryl) propanamides (8a-o)

Alkyl/aralkyl/aryl amines (**6a-o**; 0.005 mol) were dispersed in 10% Na_2CO_3 in an iodine flask (250 mL). 2-Bromopropanoyl bromide (**7**; 0.005 mol) was gradually added to the iodine flask with vigorous shaking for 3 - 5 min. The title compounds were precipitated and filtered out. The filtered out precipitates were thoroughly washed with cold distilled water and dried [10].

Synthesis of 2-({5-[(*1E,3E*)-4-(1,3-benzodioxol-5-yl)-1,3-butadienyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-(alkyl/aralkyl/aryl) propanamides (9a-o)

Compound **5** (0.001 mol) was mixed with *N,N*-dimethylformamide (8 mL) with shaking in a 50-mL round-bottom flask. The mixture was further stirred for 45 min with lithium hydride (0.001 mol) at room temperature. The synthesized electrophiles, 2-bromo-*N*-(alkyl/aralkyl/aryl) propanamides (**8a-o**), were added and the mixture again stirred for 4 - 5 h. Excess cold distilled water was poured into the mixture, which was then allowed to stand for 1 h. The *S*-substituted derivatives of **5** were obtained as solid precipitates [13].

Antimicrobial assay

Tests for antimicrobial activity were performed using the disc diffusion method of Berghe and Vlietnick [14].

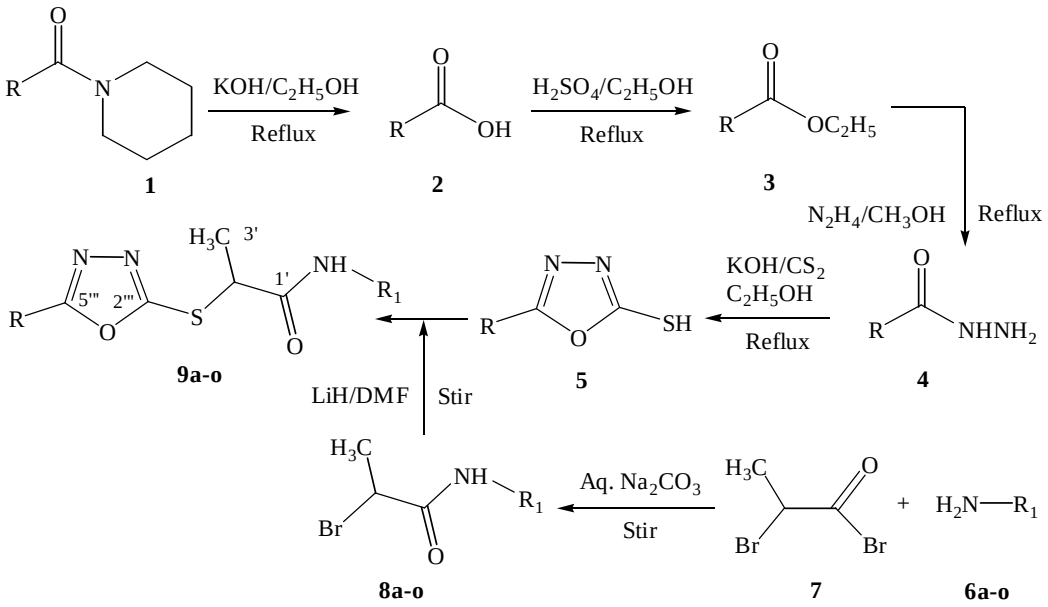
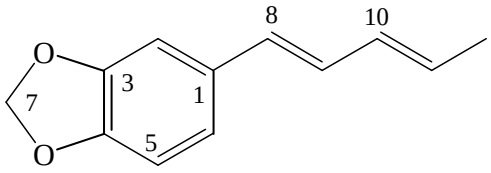
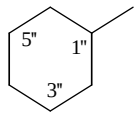
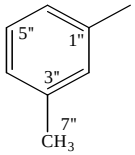
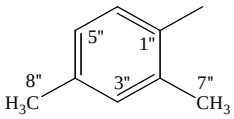
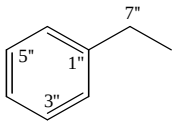
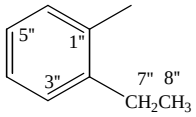
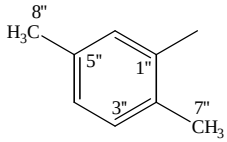
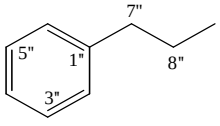
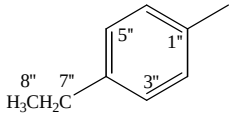
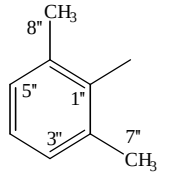
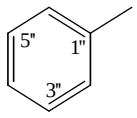
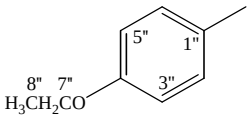
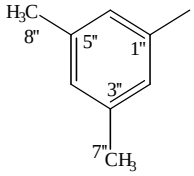
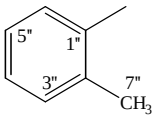
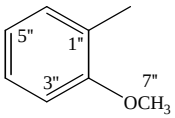
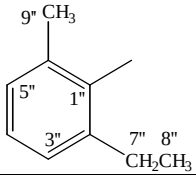


Figure 1: Protocol for the synthesis of *N*-substituted-2-({5-[(1*E*,3*E*)-4-(1,3-benzodioxol-5-yl)-1,3-butadienyl]-1,3,4-oxadiazol-2-yl}sulfanyl)propanamides (**9a-o**)

Table 1: Different R_1-groups as *N*-substituents

R =



Comp.	R_1	Comp.	R_1	Comp.	R_1
9a		**9f**		**9k**	
9b		**9g**		**9l**	
9c		**9h**		**9m**	
9d		**9i**		**9n**	
9e		**9j**		**9o**	

Antibacterial activity was assayed against *Bacillus subtilis* (JS 2004) and *Escherichia coli* (ATCC 25922), with the reference drug being rifampicin, and antifungal activity against *Aspergillus niger* (locally isolated), with the reference drug being fluconazole. These microbial strains were collected through the Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan.

Hemolytic activity assay

Hemolytic activity was performed according to Powell *et al* [15]. A blood sample was collected from a cow, and RBCs (red blood cells) were isolated. Triton X-100 and phosphate buffer saline (PBS) were employed as references.

Thrombolytic activity assay

Thrombolytic activity was assayed according to Mannan *et al* [16] using a bovine blood sample. PBS and streptokinase were employed as references. Activity was determined by the following equation, where X represents the clot weight before lysis, and Y represents the clot weight after lysis.

Inhibition (%) = [(X-Y)/X × 100

Statistical analysis

The antimicrobial data are presented as mean ± standard deviation (SD) and were analyzed by Students t-test using Microsoft Excel 2010. $P < 0.05$ was considered statistically significant.

RESULTS

Figure 1 presents the synthesis of propanamide derivatives **9a-o**. The varying alkyl/aralkyl/aryl groups are listed in Table 1.

Spectral characteristics of synthesized molecules (9a-o)

Piperine (1)

Yellow needle-like crystals; Yield: 4 %; M.P. 127-129 °C; M.F.: $C_{17}H_{19}NO_3$; M.W.: 285; IR (KBr, cm^{-1}) ν_{max}: 2950, 1650, 1620, 1600, 1250, 1140, 1005; ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 7.42-7.34 (m, 1H, H-10), 6.98 (d, J = 1.5 Hz, 1H, H-2), 6.89 (dd, J = 1.5, 7.8 Hz, 1H, H-6), 6.78-6.64 (m, 3H, H-5,8,9), 6.45 (d, J =15.3 Hz, 1H, H-11), 5.98 (s, 2H, H-7), 3.59-3.56 (m, 4H, H-2',6'), 1.68-1.61 (m, 6H, H-3',4',5'); EIMS (*m/z*): 285 (M^+, 60%), 202 (28%), 201 (100%), 174 (23%), 173 (44%), 115 (93%).

Piperic acid (2)

Light yellow crystals; Yield: 74 %; M.P. 217-218 °C; M.F.: $C_{12}H_{10}O_4$; M.W.: 218; IR (KBr, cm^{-1}) ν_{max}: 3217, 1672, 1599, 1257,1148, 1036; ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.44-7.35 (m, 1H, H-10), 6.97 (d, J = 1.5 Hz, 1H, H-2), 6.89 (dd, J = 1.5, 7.8 Hz, 1H, H-6), 6.77-6.63 (m, 3H, H-5,8,9), 5.96 (s, 2H, H-7), 5.92 (d, J =15.3 Hz, 1H, H-11); EIMS (*m/z*): 218 (M^+, 57%), 202 (27%), 201 (100%), 174 (23%), 173 (44%), 115 (93%).

Ethyl piperate (3)

Light yellow crystals; Yield: 64 %; M.P. 132-134 °C; M.F.: $C_{14}H_{14}O_4$; M.W.: 246; IR (KBr, cm^{-1}) ν_{max}: 2953, 2923, 1704, 1608, 1234,1134, 997; ^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.43-7.34 (m, 1H, H-10), 6.97 (d, J = 1.5 Hz, 1H, H-2),.6.89 (dd, J = 1.5, 7.8 Hz, 1H, H-6), 6.78-6.63 (m, 3H, H-5,8,9), 5.96 (s, 2H, H-7), 5.92 (d, J = 15.3 Hz,1H, H-11), 4.20 (q, J = 7.2 Hz, 2H, $O\underline{CH_2}CH_3$), 1.29 (t, J = 7.2 Hz, 3H, $OCH_2\underline{CH_3}$); EIMS (*m/z*): 246 (M^+, 60%), 202 (29%), 201 (100%), 174 (22%), 173 (43%), 115 (92%).

Piperic carbohydrazide (4)

Yellowish orange crystals; Yield: 72 %; M.P. 149-150 °C; M.F.: $C_{12}H_{12}N_2O_3$; M.W.: 232; IR (KBr, cm^{-1}) ν_{max}: 3317, 2918, 2849, 1658, 1488, 1248, 1038, 991; ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 7.12-7.05 (m, 1H, H-10), 6.97 (d, J = 1.5 Hz, 1H, H-2), 6.90 (dd, J = 1.5, 7.8 Hz, 1H, H-6), 6.78-6.69 (m, 3H, H-5,8,9), 5.98 (s, 2H, H-7), 5.94 (d, J = 15.6 Hz,1H, H-11); EIMS (*m/z*): 232 (M^+, 23%), 167 (42%), 149 (100%), 57 (38%), 71 (24%), 43 (20%).

5-[(*1E,3E*)-4-(1,3-Benzodioxol-5-yl)-1,3-butadienyl]-1,3,4-oxadiazol-2-thiol (5)

Bright yellowish orange crystals; Yield: 75 %; M.P. 215-217 °C; M.F.: $C_{13}H_{10}N_2O_3S$; M.W.: 274; IR (KBr, cm^{-1}) ν_{max}: 2935, 2849, 2536, 1655, 1537, 1250,1144, 1034; ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 7.30-7.24 (m, 1H, H-10), 6.97 (s, 1H, H-2), 6.90 (d, J = 8.0 Hz, 1H, H-6), 6.78-6.73 (m, 3H, H-5,8,9), 5.97-5.96 (m, 3H, H-7,11); EIMS (*m/z*): 215 (5.8%), 201 (20%), 199 (20%), 148 (37%), 55 (100%).

2-({5-[(*1E,3E*)-4-(1,3-Benzodioxol-5-yl)-1,3-butadienyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-cyclohexylpropanamide (9a)

Light yellow crystals; Yield: 70 %; M.P. 111-112 °C; M.F.: $C_{22}H_{25}N_3O_4S$; M.W.: 427; IR (KBr, cm^{-1}) ν_{max}: 3250, 2930, 2830, 1670, 1645, 1490, 1250,

1045, 980; ^{1}H-NMR (300 MHz, $CDCl_3$, *δ*/ppm): 7.29-7.25 (m, 1H, H-10). 7.01 (d, *J* = 1.2 Hz, 1H, H-2), 6.93 (dd, *J* = 8.1, 1.5 Hz, 1H, H-6), 6.84-6.73 (m, 3H, H-5,8,9), 6.48 (d, *J* = 15.6 Hz, 1H, H-11), 6.01 (s, 2H, H-7), 3.80 (br.s, 1H, 2'), 3.32 (s, 1H, H-1"), 1.77 (d, *J* = 8.1 Hz, 3H, H-3'), 1.55-1.16 (m, 10H, H-2" to H-6"); EIMS (*m/z*): 427 (M^+), 167 (40%), 149 (100%), 57 (36%), 71 (24%), 43 (20%).

2-({5-[(*1E,3E*)-4-(1,3-Benzodioxol-5-yl)-1,3-butadienyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-benzylpropanamide (9b)

Yellowish brown crystals; Yield: 75 %; M.P. 114-115 °C; M.F.: $C_{23}H_{21}N_3O_4S$; M.W.: 435; IR (KBr, cm^{-1}) v_{max}: 3250, 2920, 2825, 1673, 1650, 1440, 1250, 1040, 970; ^{1}H-NMR (300 MHz, $CDCl_3$, *δ*/ppm): 7.41-7.33 (m, 5H, H-2" to H-6"),7.21-7.19 (m, 1H, H-10), 6.97 (d, *J* = 1.5 Hz, 1H, H-2), 6.89 (dd, *J* = 8.1, 1.5 Hz, 1H, H-6), 6.82-6.60 (m, 3H, H-5,8,9), 6.35 (d, *J* = 14.6 Hz, 1H, H-11), 6.01 (s, 2H, H-7), 4.43 (s, 2H, H-7"), 4.22-4.36 (m, 1H, H-2'), 1.76 (d, *J* = 8.1 Hz, 3H, H-3'); EIMS (*m/z*): 435 (M^+), 167 (41%), 149 (100%), 57 (35%), 71 (22%), 43 (20%).

2-({5-[(*1E,3E*)-4-(1,3-Benzodioxol-5-yl)-1,3-butadienyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-(phenylethyl)propanamide (9c)

Light yellow crystals; Yield: 73 %; M.P. 115-116 °C; M.F.: $C_{24}H_{23}N_3O_4S$; M.W.: 449; IR (KBr, cm^{-1}) v_{max}: 3340, 2980, 2805, 1675, 1655, 1450, 1250, 1045, 990; ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 7.78-7.54 (m, 2H, H-3",5"), 7.33-7.21 (m, 3H, H-2 " , 4" & H-6"), 7.03-6.75 (m, 7H, H-2,5,6,8,9,10,11), 6.00 (s, 2H, H-7), 4.10-4.04 (m, 1H, H-2'), 3.04 (t, *J* = 8.1 Hz, 2H, H-8"), 2.81 (t, *J* = 7.5 Hz, 2H, H-7"), 1.59 (d, *J* = 8.1 Hz, 3H, H-3'); EIMS (*m/z*): 449 (M^+), 167 (40%), 149 (100%), 57 (35%), 71 (22%), 43 (20%).

2-({5-[(*1E,3E*)-4-(1,3-Benzodioxol-5-yl)-1,3-butadienyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-phenylpropanamide (9d)

Light yellow crystals; Yield: 65 %; M.P. 110-111 °C; M.F.: $C_{22}H_{19}N_3O_4S$; M.W.: 421; IR (KBr, cm^{-1}) v_{max}: 3330, 2980, 2875, 1674, 1661, 1489, 1250, 1045, 980; ^{1}H-NMR (300 MHz, $CDCl_3$, *δ*/ppm):7.41-7.33 (m, 5H, H-2" to 6"),7.21-6.98 (m, 2H, H-2,10), 6.98 (d, *J* = 8.1 Hz, 1H, H-6), 6.91-6.74 (m, 3H, H-5,8,9), 6.34 (d, *J* = 14.6 Hz, 1H, H-11), 6.01 (s, 2H, H-7), 4.37-4.24 (m, 1H, H-2'), 1.79 (d, *J* = 8.1 Hz, 3H, H-3'); EIMS (*m/z*): 421 (M^+), 167 (42%), 149 (100%), 57 (37%), 71 (22%), 43 (21%).

2-({5-[(*1E,3E*)-4-(1,3-Benzodioxol-5-yl)-1,3-butadienyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-(2-methylphenyl)propanamide (9e)

Light brown crystals; Yield: 70 %; M.P. 168-169 °C; M.F.: $C_{23}H_{21}N_3O_4S$; M.W.: 435; IR (KBr, cm^{-1}) v_{max}: 3350, 2985, 2875, 1678, 1651, 1497, 1250, 1045, 990; ^{1}H-NMR (300 MHz, $CDCl_3$, *δ*/ppm): 7.79 (d, *J* = 8.2 Hz, 1H, 6"),7.24 (d, *J* = 7.8 Hz, 1H, H-3"), 7.19 (t, *J* = 7.8 Hz, 1H, H-5"), 7.15 (t, *J* = 7.8 Hz, 1H, H-4"), 7.13-7.09 (m, 1H, H-10), 7.08-7.02 (m, 1H, H-2), 6.92 (d, *J* = 8.1 Hz, 1H, H-6), 6.81-6.77 (m, 3H, H-5,8,9), 6.40-6.34 (m, 1H, H-11), 6.01 (s, 2H, H-7), 4.39-4.33 (m, 1H, H-2'), 2.41 (s, 3H, H-7"), 1.82 (d, *J* = 6.9 Hz, 3H, H-3'); EIMS (*m/z*): 435 (M^+), 167 (40%), 149 (100%), 57 (35%), 71 (22%), 43 (20%).

2-({5-[(*1E,3E*)-4-(1,3-Benzodioxol-5-yl)-1,3-butadienyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-(3-methylphenyl)propanamide (9f)

Dark brownish yellow crystals; Yield: 70 %; M.P. 190-191 °C; M.F.: $C_{23}H_{21}N_3O_4S$; M.W.: 435; IR (KBr, cm^{-1}) v_{max}: 3555, 2980, 2875, 1672, 1645, 1489, 1250, 1045, 995; ^{1}H-NMR (300 MHz, $CDCl_3$, *δ*/ppm): 7.59 (d, *J* = 8.4 Hz, 1H, H-6"), 7.34 (s, 1H, H-2"), 7.15 (t, *J* = 8.1 Hz, 1H, H-5"), 7.05 (t, *J* = 7.8 Hz, 1H, H-4"), 7.13-7.09 (m, 1H, H-10), 7.08-7.02 (m, 1H, H-2), 6.92 (d, *J* = 8.1 Hz, 1H, H-6), 6.81-6.77 (m, 3H, H-5,8,9), 6.40-6.34 (m, 1H, H-11), 6.01 (s, 2H, H-7), 4.39-4.33 (m, 1H, H-2'), 2.36 (s, 3H, CH_3-7"), 1.82 (d, *J* = 6.9 Hz, 3H, H-3'); EIMS (*m/z*): 435 (M^+), 167 (40%), 149 (100%), 57 (35%), 71 (22%), 43 (20%).

2-({5-[(*1E,3E*)-4-(1,3-Benzodioxol-5-yl)-1,3-butadienyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-(2-ethylphenyl)propanamide (9g)

Bright yellow crystals; Yield: 69 %; M.P. 122-123 °C; M.F.: $C_{24}H_{23}N_3O_4S$; M.W.: 449; IR (KBr, cm^{-1}) v_{max}: 3350, 2985, 2875, 1682, 1656, 1479, 1250, 1045, 990; ^{1}H-NMR (300 MHz, $CDCl_3$, *δ*/ppm): 7.23 (dd, *J* = 8.2, 2.8 Hz, 1H, H-6"), 7.21-7.17 (m, 1H, H-10), 7.18 (dt, *J* = 8.2, 2.6 Hz, 1H, H-5"), 7.09 (dt, *J* = 8.2, 2.8 Hz, 1H, H-4"), 7.08 (d, *J* = 1.5 Hz, 1H, H-2), 7.03 (dd, *J* = 8.2, 2.8 Hz, 1H, H-3"), 6.91 (dd, *J* = 8.1, 1.5 Hz, 1H, H-6), 6.88-6.62 (m, 3H, H-5,8,9), 6.37 (d, *J* = 14.6 Hz, 1H, H-11), 5.98 (s, 2H, H-7), 4.35-4.25 (m, 1H, H-2'), 2.75 (q, *J* = 7.9 Hz, 2H, H-7"), 2.13 (t, *J* = 7.8 Hz, 3H, H-8"), 1.81 (d, *J* = 8.1 Hz, 3H, H-3'); EIMS (*m/z*): 449 (M^+), 167 (40%), 149 (100%), 57 (35%), 71 (22%), 43 (20%).

2-({5-[(*1E,3E*)-4-(1,3-Benzodioxol-5-yl)-1,3-butadienyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-(4-ethylphenyl)propanamide (9h)

Light yellowish crystals; Yield: 69 %; M.P. 167-168 °C; M.F.: $C_{24}H_{23}N_3O_4S$; M.W.: 449; IR (KBr, cm^{-1}) ν_{max}: 3250, 2935, 2850, 1675, 1650, 1478, 1250, 1030, 980; ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 7.56-7.30 (m, 4H, H-2",3",5",6"), 7.21-7.18 (m, 1H, H-10), 6.99 (d, *J* = 1.5 Hz, 1H, H-2), 6.89 (dd, *J* = 8.1, 1.5 Hz, 1H, H-6), 6.82-6.60 (m, 3H, H-5,8,9), 6.35 (d, *J* = 14.6 Hz, 1H, H-11), 6.00 (s, 2H, H-7), 4.36-4.22 (m, 1H, H-2'), 2.75 (q, *J* = 7.9 Hz, 2H, H-7"), 2.11 (t, *J* = 7.8 Hz, 3H, H-8"), 1.79 (d, *J* = 8.1 Hz, 3H, H-3'); EIMS (*m/z*): 449 (M^+), 167 (40%), 149 (100%), 57 (35%), 71 (22%), 43 (20%).

2-({5-[(*1E,3E*)-4-(1,3-Benzodioxol-5-yl)-1,3-butadienyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-(4-ethoxyphenyl)propanamide (9i)

Dark brownish crystals; Yield: 66 %; M.P. 151-152 °C; M.F.: $C_{24}H_{23}N_3O_5S$; M.W.: 465; IR (KBr, cm^{-1}) ν_{max}: 3250, 2935, 2850, 1671, 1655, 1480, 1250, 1040, 980; ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 7.60-7.35 (m, 4H, H-2",3",5",6"), 7.22-7.18 (m, 1H, H-10), 6.98 (d, *J* = 1.5 Hz, 1H, H-2), 6.90 (dd, *J* = 8.1, 1.5 Hz, 1H, H-6), 6.85-6.61 (m, 3H, H-5,8,9), 6.37 (d, *J* = 14.4 Hz, 1H, H-11), 5.99 (s, 2H, H-7), 4.33-4.24 (m, 1H, H-2'), 3.99 (q, *J* = 7.4 Hz, 2H, H-7"), 1.78 (d, *J* = 8.1 Hz, 3H, H-3'), 1.32 (t, *J* = 7.4 Hz, 3H, H-8"); EIMS (*m/z*): 465 (M^+), 167 (40%), 149 (100%), 57 (35%), 71 (22%), 43 (20%).

2-({5-[(*1E,3E*)-4-(1,3-Benzodioxol-5-yl)-1,3-butadienyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-(2-methoxyphenyl)propanamide (9j)

Yellowish brown crystals; Yield: 75 %; M.P. 198-199 °C; M.F.: $C_{23}H_{21}N_3O_5S$; M.W.: 451; IR (KBr, cm^{-1}) ν_{max}: 3350, 2985, 2875, 1684, 1667, 1480, 1250, 1045, 990; ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 8.20 (d, *J* = 7.6 Hz, 1H, H-6"), 7.24-7.20 (m, 1H, H-10), 7.07 (t, *J* = 7.2 Hz, 1H, H-5"),7.04 (t, *J* = 7.6 Hz, 1H, H-4"), 6.96 (d, *J* = 8.0 Hz, 1H, H-3"), 6.92 (d, *J* = 1.5 Hz, 1H, H-2), 6.88 (dd, *J* = 8.1, 1.5 Hz, 1H, H-6), 6.87-6.63 (m, 3H, H-5,8,9), 6.36 (d, *J* = 14.6 Hz, 1H, H-11), 5.99 (s, 2H, H-7), 4.36-4.29 (m, 1H, H-2'), 3.80 (s, 3H, H-7"), 1.82 (d, *J* = 8.1 Hz, 3H, H-3'); EIMS (*m/z*): 451 (M^+),167 (40%), 149 (100%), 57 (35%), 71 (22%), 43 (20%).

2-({5-[(*1E,3E*)-4-(1,3-Benzodioxol-5-yl)-1,3-butadienyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-(2,4-dimethylphenyl)propanamide (9k)

Light yellow crystals; Yield: 69 %; M.P. 158-159 °C; M.F.: $C_{24}H_{23}N_3O_4S$; M.W.: 449; IR (KBr, cm^{-1}) ν_{max}: 3497, 2985, 2870, 1678, 1670, 1485, 1250, 1045, 990; ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 7.68 (d, *J* = 8.2 Hz, 1H, H-6"),7.14-7.09 (m, 1H, H-10), 7.05 (d, *J* = 8.2 Hz, 1H, H-5"),6.95 (s, 1H, H-3"),6.92 (d, *J* = 1.4 Hz, 1H, H-2), 6.91 (d, *J* = 8.1, 1H, H-6), 6.77-6.63 (m, 3H, H-5,8,9), 6.36 (d, *J* = 14.6 Hz, 1H, H-11), 6.01 (s, 2H, H-7), 4.36-4.30 (m, 1H, H-2'), 2.21 (s, 6H, H-7", 8"), 1.81 (d, *J* = 6.9 Hz, 3H, H-3'); EIMS (*m/z*): 449 (M^+), 167 (40%), 149 (100%), 57 (35%), 71 (22%), 43 (20%).

2-({5-[(*1E,3E*)-4-(1,3-Benzodioxol-5-yl)-1,3-butadienyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-(2,5-dimethylphenyl)propanamide (9l)

Light yellow solid; Yield: 71 %; M.P. 141-142 °C; M.F.: $C_{24}H_{23}N_3O_4S$; M.W.: 449; IR (KBr, cm^{-1}) ν_{max}: 3250, 2930, 2850, 1673, 1664, 1480, 1250, 1040, 980; ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 7.19 (s, 1H, H-6"), 7.08-7.15 (m, 1H, H-10), 7.04 (d, *J* = 8.0 Hz, 1H, H-3"), 6.94 (d, *J* = 8.0 Hz, 1H, H -4"), 6.92 (s, 1H, H-2), 6.91 (d, *J* = 8.1, 1H, H-6), 6.78-6.83 (m, 2H, H-5,8), 6.66-6.58 (m, 1H, H-9), 6.35-6.31 (m, 1H, H-11), 6.00 (s, 2H, H-7), 4.36-4.31 (m, 1H, H-2'), 2.41 (s, 3H, H-7"), 2.24 (s, 3H, H-8"), 1.81 (d, *J* = 6.9 Hz, 3H, H-3'); EIMS (*m/z*): 449 (M^+), 167 (41%), 149 (100%), 57 (35%), 71 (22%), 43 (20%).

2-({5-[(*1E,3E*)-4-(1,3-Benzodioxol-5-yl)-1,3-butadienyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-(2,6-dimethylphenyl)propanamide (9m)

Yellowish brown crystals; Yield: 69 %; M.P. 167-168 °C; M.F.: $C_{24}H_{23}N_3O_4S$; M.W.: 449; IR (KBr, cm^{-1}) ν_{max}: 3290, 2920, 2825, 1677, 1657, 1477, 1250, 1040, 980; ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 7.15-7.11 (m, 1H, H-10), 7.10-7.07 (m, 3H, H-3" to 5"), 6.94 (s, 1H, H-2), 6.92 (d, *J* = 8.1 Hz, 1H, H-6), 6.81-6.67 (m, 3H, H-5,8,9), 6.34-6.29 (m, 1H, H-11), 6.02 (s, 2H, H-7), 4.31-4.29 (m, 1H, H-2'), 2.46 (s, 6H, H-7",8"), 1.80 (d, *J* = 6.9 Hz, 3H, H-3'); EIMS (*m/z*):449 (M^+), 167 (40%), 149 (100%), 57 (35%), 71 (22%), 43 (20%).

2-({5-[(*1E,3E*)-4-(1,3-Benzodioxol-5-yl)-1,3-butadienyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-(3,5-dimethylphenyl)propanamide (9n)

Light brown crystals; Yield: 79 %; M.P. 143-144 °C; M.F.: $C_{24}H_{23}N_3O_4S$; M.W.: 449; IR (KBr, cm^{-1}) ν_{max}: 3350, 2985, 2875, 1678, 1659, 1476, 1250, 1045, 990; ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 7.13-7.09 (m, 1H, H-10), 6.97-6.93 (m, 2H, H-2,4"), 7.52-7.42 (m, 2H, H-2",6"), 6.90 (d, *J* = 8.1 Hz, 1H, H-6), 6.81-6.77 (m, 2H, H-5,8), 6.66-6.61 (m, 1H, H-9), 6.40-6.34 (m, 1H, H-11), 6.00 (s,

2H, H-7), 4.35-4.33 (m, 1H, H-2'), 2.40 (s, 6H, H-7",8"), 1.80 (d, *J* = 6.9 Hz, 3H, H-3'); EIMS (*m/z*): 167 (40%), 149 (100%), 57 (35%), 71 (22%), 43 (20%).

2-({5-[(*1E,3E*)-4-(1,3-Benzodioxol-5-yl)-1,3-butadienyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-(2-ethyl-6-methylphenyl)propanamide (9o)

Light yellow crystals; Yield: 69 %; M.P. 120-121 °C; M.F.: $C_{25}H_{25}N_3O_4S$; M.W.: 463; IR (KBr, cm^{-1}) ν_{max}: 3350, 2985, 2875, 1681, 1669, 1498, 1250, 1045, 990; ^{1}H-NMR (300 MHz, $CDCl_3$, *δ*/ppm): 7.17-7.00 (m, 3H, H-3" to 5"), 7.15-7.08 (m, 1H, H-10), 6.97 (s 1H, H-2), 6.92 (d, *J* = 8.1 Hz, 1H, H-6), 6.81-6.78 (m, 2H, H-5,8), 6.67-6.65 (m, 1H, H-9), 6.37-6.34 (m, 1H, H-11), 6.01 (s, 2H, H-7), 4.33-4.31 (m, 1H, H-2'), 2.49 (q, *J* = 7.6 Hz, 2H, H-7"), 1.99 (s, 3H, H-9"), 1.81 (d, *J* = 6.9 Hz, 3H, H-3'), 1.05 (t, *J* = 7.6 Hz, 3H, H-8"); EIMS (*m/z*): 463 (30%) [M^+], 167 (40%), 149 (100%), 57 (35%), 71 (22%), 43 (20%).

Antibacterial activity

Table 2 and Table 3 show the results for antimicrobial, hemolytic and thrombolytic activities. The target compounds were more active antimicrobial agents than the alkaloid piperine. Compounds **9a** and **9d** bearing cyclohexyl and phenyl groups, respectively, exhibited potent inhibition activity.

DISCUSSION

Compound, **9n**, has been selected for single compound discussion. The EIMS spectrum and proton integration in ^{1}H-NMR spectrum helped to determine its molecular formula and molecular mass. IR spectra showed prominent absorption bands (cm^{-1}) at 3350, 2985, 2875, 1659, 1678, 1600-1450, 1250 and 1045 for N-H, C-H (symmetric and asymmetric), C=N and NC=O, respectively, which are indicative of aromatic systems and methylenedioxy (symmetric and asymmetric) functionalities.

In the aliphatic region of ^{1}H-NMR, two signals as doublet and multiplet at *δ* 4.35-4.33 (m, 1H, H-2') and 1.80 (d, *J* = 6.9 Hz, 3H, H-3') supported the presence of a propanamoyl group. A singlet at *δ* 2.40 (s, 6H, H-7",8") was ascribed to two methyl groups linked to benzene ring. The two methylenic protons were shown by a singlet at *δ* 6.00 (s, 2H, H-7). The remaining part of the molecule was justified by the signals in the aromatic and olefinic region at *δ* 7.13-7.09 (m, 1H, H-10), 6.97-6.93 (m, 2H, H-2,4"), 7.52-7.42 (m, 2H, H-2",6"), 6.90 (d, *J* = 8.1 Hz, 1H, H-6), 6.81-6.77 (m, 2H, H-5,8), 6.66-6.61 (m, 1H, H-9) and 6.40-6.34 (m, 1H, H-11).

Table 2: Zone of inhibition (mm) for antimicrobial activity of **1-5** and **9a-o**

Compound	**Zone of inhibition (mm)**		
	Antibacterial activity		**Antifungal activity**
	Bacillus subtilis	***Escherichia coli***	***Aspergilus niger***
1	-	-	-
2	-	-	-
3	-	-	-
4	-	-	-
5	7	9	7
9a	14	15	18
9b	12	9	8
9c	12	11	14
9d	16	16	17
9e	7	9	7
9f	10	8	9
9g	15	17	13
9h	-	-	-
9i	13	10	12
9j	-	-	-
9k	12	12	10
9l	-	-	-
9m	14	15	12
9n	-	-	-
9o	10	11	10
Rifampicin	**21**	**23**	-
Fluconazole	-	-	**19**

Table 3: Hemolytic and thrombolytic activities of **1-5** and **9a-o**

Compound	Hemolytic activity (%)	Thrombolytic activity (%)
1	19.27	9.67
2	10.13	13.16
3	13.58	3.45
4	2.55	12.43
5	11.62	1.32
9a	42.99	4.19
9b	14.06	4.85
9c	15.85	25.56
9d	41.93	31.49
9e	49.61	39.54
9f	45.55	35.34
9g	42.99	22.54
9h	20.71	25.61
9i	39.38	33.78
9j	74.73	7.53
9k	15.50	10.30
9l	21.71	14.63
9m	1.00	1.43
9n	7.92	17.32
9o	41.81	21.65
Triton X-100	**98.74**	-
PBS	**0.5**	**0.5**
Streptokinase	-	**85.64**

The absence of a molecular ion peak indicated instability of the compound in the mass spectrometer. The base peak appeared at *m/z* 149 due to a 1,3-benzodioxol-5-ylethane cation. The other prominent peaks at 167 and 57 were for 2-(5-ethyl-1,3,4-oxadiazolidin-2-ylthio)ethenone and 3-methyl-1,2-oxazirene, respectively. Thus, compound **9n** was named 2-({5-[(1*E*,3*E*)-4-(1,3-benzodioxol-5-yl)-1,3-butadienyl]-1,3,4-oxadiazol-2-yl}sulfanyl)-*N*-(3,5-dimethylphenyl) propanamide.

Antimicrobial activity

The results proved that the inclusion of the 1,3,4-oxadiazole and propanamide functionality enhanced the antimicrobial behavior of the compounds. The initial compounds **1-4** showed no activity at all; however, **5** with a 1,3,4-oxadiazole ring exhibited some zone inhibition and target molecules were more bioactive. Compounds **9a**, **9d**, **9g** and **9m** exhibited better activity against *B. substilis* (Gram positive) with zone inhibition values of 14, 16, 15 and 14 mm, respectively, compared to the reference drug with 21 mm. The same four compounds showed better results against *E. coli* (Gram negative) with zone inhibition values of 15, 16, 17 and 15 mm, respectively, compared to the reference drug with 23 mm. Against *A. niger*, compounds **9a**, **9c** and **9d** had the highest activity with zone inhibition values of 18, 14 and 17, respectively, compared to the reference drug with 19 mm.

Hemolytic and thrombolytic activities

Among the list of compounds, **4**, **9m** and **9n** showed the least toxicity. Compounds **9e** and **9f** showed the highest toxicity. Moderate toxicity was exhibited by compounds **9b**, **9c**, **9h**, **9k** and **9l**. Those compounds that showed low or moderate toxicity could be used in a drug discovery program. With regard to thrombolytic activity, piperine showed a low clot lysis percentage, which increased when the piperine nucleus was modified, creating the different compounds **9a-o**. Compounds **9d**, **9e, 9f** and **9i** were found to be the most active ones.

CONCLUSION

Compounds **9a**, **9d**, **9g** and **9m** possess good antibacterial and antifungal activities. Some of the synthesized compounds also exhibit some level of hemolytic and thrombolytic activities, and have shown enough potentials to be considered for further studies.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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