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Synthesis and Biological Studies of N-Phenyl Substituted 2-(-5-(pyridine-4-yl)-1,3,4-oxadiazole-2-yl thio)acetamides

M.E. Bhanoji Rao and Vikas G. Rajurkar*

Department of Pharmaceutical Chemistry, Roland Institute of Pharmaceutical Sciences, Ambapua, Khodasingi, Berharmpur-760 010, India

*Corresponding author: E-mail: vikas_rajurkar_1973@yahoo.co.in

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As oxadiazole have proven to be good antimicrobial agents, Antitubercular, analgesic (peripheral and central) and antiinflammatory agents. A new series of oxadiazole derivatives were synthesized and characterized by ^{1}H NMR, IR, GCMS sophisticated analytical instruments and were evaluated for their antimicrobial activity, antitubercular activity, toxicity as per standard guidelines, analgesic (peripheral and central) and antiinflammatory activity. Out of several derivatives synthesized a few of N-phenyl substituted 2-(5-(pyridine-4-yl)-1,3,4-oxadiazole-2-yl thio)acetamide explores good antimicrobial activity by using Cup-Plate method, antitubercular activity by middle brook 7H9 agar medium against $H_{37}Rv$, analgesic activity by Writhing and Tail immersion method and antiinflammatory by rat paw edema method.

Key Words: 1,3,4-Oxadiazole derivatives, Antimicrobial, Anti T.B., Toxicity study, Analgesic and antiInflammatory activity.

INTRODUCTION

Microbial infections are most common infections and commonly used antibacterials, antifungals are antibiotics and recently used halogenated quinolones, azoles and other synthetic agents are being used to combat the newer and resistant microbial infections. Azoles in the particular 1,3,4-oxadiazoles have shown significant antimicrobial activity[1-4], which is practically 1,3,4-oxadiazoles have been the basis for the most of antifungals. Compounds with the 2-thia substituted were also shown to possess biological activity such as anti-fungal, antibacterial[1] and antiinflammatory activity. Here, an attempt is made to synthesize of N-phenyl substituted 2-(-5-(pyridine-4-yl)-1,3,4-oxadiazole-2-yl thio)acetamide as potent antimicrobial agents, antituberculasis analgesic and antiinflammatory agents.

EXPERIMENTAL

All chemicals were used after purification and supplied by Loba Chemie, Qualigens & Research Labs. Melting points of newly synthesized compounds were determined in open capillary tube and were found uncorrected. The structure of the synthesized compounds were confirmed by spectral data. The IR spectra were recorded on FTIR 8400 F-Shimadzu spectrometer using KBr disc pellet method. ^{1}H NMR spectra were recorded on AVANCE II 400 and Verian Mercury ^{1}H 300 using DMSO and $CDCl_3$ as solvent. GC mass spectra were recorded on GCMS-QP-5050 Shimadzu.

Synthesis of isonicotinyl hydrazide (1): In round bottom flask 4-pyridine carboxylic acid (0.01 mol) and hydrazine hydrate 99 % (0.01 mol) was taken along with alcohol and the mixture was refluxed for 4 h. Then from the reaction mixture alcohol was removed under reduced pressure. Solid residue was obtained, recrystallized with ethanol. Molecular formula $C_6H_7N_3O$, yield 55 % and m.p. 172 ºC.

Synthesis of 5-(pyridine-4-yl)-1,3,4-oxadiazol-2-thiol (2): To a solution of isonicotinyl hydrazide (**1**) (13.7 g, 0.1 mol) in ethanol was added a solution of potassium hydroxide (5.6 g, 0.1 mol) in water (36 mL) and stirred well. Carbon disulfide (7 mL) was then added and refluxed till the evolution of H_2S gas ceased. Excess of solvent was removed and residue poured into ice- cold water (100 mL). It was filtered to remove suspended impurities and acidified with dil HCl to obtain the desired product. It was then filtered, washed with cold water and recrystallized from ethanol to get yellowish crystals. Yield 65 %, m.p. 256 ºC.

Synthesis of N-substituted α-chloroacetanilides (3)

General procedure for aromatic amines: The aromatic amines (0.05 mol) were dissolved in a mixture of glacial acetic acid (25 mL) and saturated solution of sodium acetate (25 mL) and cooled to 5 ºC. To this, chloro acetyl chloride (6.2 mL, 0.075 mol) was added dropwise at 0-5 ºC under constant stirring. It was left at room temperature for 5-6 h and the crude product that separated was filtered, washed with 50 %

acetic acid and cold water. It was recrystallized from suitable solvents.

Synthesis of N-phenyl substituted 2-(-5-(pyridine-4-yl)-1,3,4-oxadiazole-2-yl thio) acetamide (4): Compound **2** (1.79 g, 0.1 mol) was dissolved in aqueous potassium hydroxide (0.61 g, 10 mL water) under stirring till a clear yellow solution was obtained. It was filtered to remove any suspended impurities. Then various aromatic N-substituted α-chloroacetanilides (0.11 mol) were added in small portions with stirring at 50-60 ºC for 4-5 h. The reaction mixture was left overnight. On next day, the precipitate was filtered, washed with cold water, dried and recrystallized from ethanol (**Scheme-I**) (Table-1).

Albino mice of either sex (weighing 20-30 g) were used for toxicity and analgesic activity, respectively. The animals were housed in standard laboratory conditions with natural light and dark place cycle. They were fed on standard pellets and *ad libitum*. Animals were acclimatized to their environment for 1 week prior to experimentation.

Toxicity studies[5]: Healthy swiss albino mice weighing between 20-30 g were used in the present investigation. The samples were tested from 500 mg to 3000 mg/kg body weight (as suspended in propylene glycol) in groups of 6 animals by Intraperitoneal rout administration. The control group of animals received only the vehicle (propylene glycol). The animals were observed for 48 h after the time of administration of test compound to record the mortality.

Analgesic activity

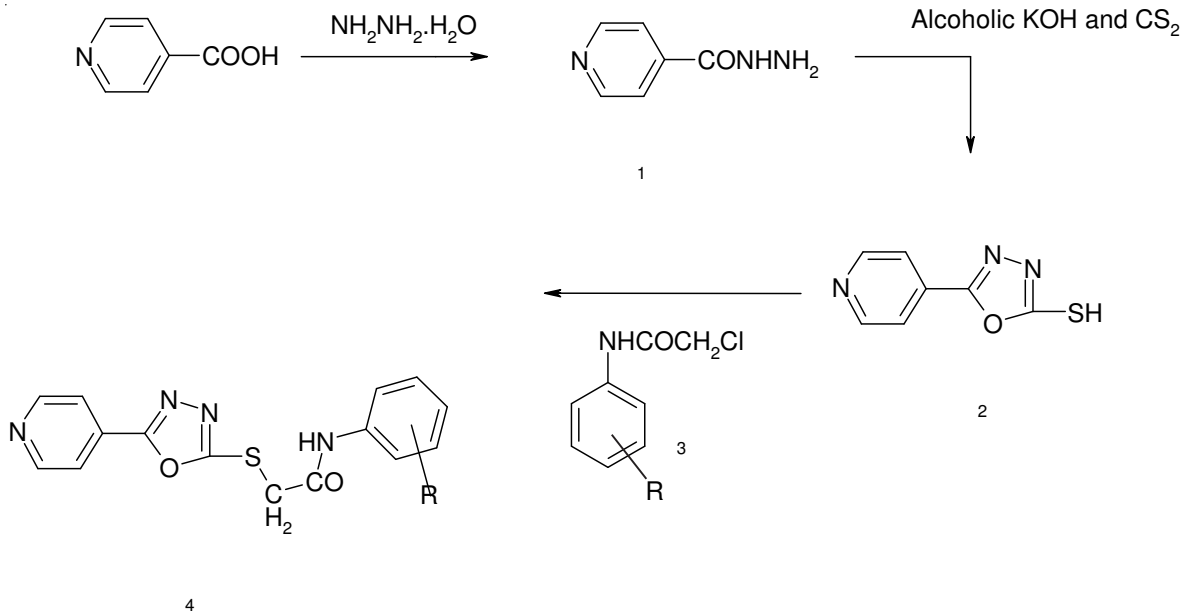
Acetic acid induced writhing method: The test compounds and diclofenac at dose of 1/10th of LD_{50} and 10 mg/kg body weight were administred in the suspension form intraperitoneally to respective mice groups (6 animals in each group) 0.5 h before writhing induction. Mice in control group received 10 mL/kg body weight volume of vehicle. Writhing was induced in mice by an injection of 0.6 % aqueous acetic acid (10 mL/kg body weight). Number of writhing episodes occurring between 5 and 15 min after acetic acid injection was recorded.

Tail immersion method: The mice were selected by immersing the tail in hot water at temperature 55 ± 5 ºC and the base reaction time was noted. The animals that showed positive response within 5 s (withdrawal of tail clearly out of water) were selected. The test compounds 1/10th of LD_{50} dose were administred in the suspension form intrapenitoneally to respective mice groups (6 animals in each groups), pentazocine 5 mg/kg body weight were administred intraperitoneally to standard mice group. The mice in control group received propylene glycol 10 mL/kg body weight as control vehicle. Observations were made upto 3 h after administration the test compounds and standard drug and the perce protection was calculated.

Antiinflammatory activity: Edema was induced in rats by injecting carrageenan (0.1 mL) into the subplantor region of the right hind paw of each rat. The test compounds and ibuprofen were administred at a dose 1/10th of the LD_{50} per kg body weight intraperitoneally to respective groups of 6 rats in each, 0.5 h before carrageenan injection. Rats in control received an equal volume of vehicle. The volume of edema was measured by plethysmometer at 0, 1, 2, 3 and 4 h after carrageenan injection.

Antimicrobial activity: The antimicrobial activity was carried out by using Cup-Plate method[6,7] by using microbial strains as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aerugenosa* and *Klebsiella pneumonia* with incubation period of 24 h at temperature 37 ºC. The standard drug used was norfloxacin (50 µg/0.1 mL) and the test compounds at concentrations of 200 and 400 µg/0.1 mL.

Antifungal activity was carried out using Cup-Plate method[5,7]. By using fungal strains as *Aspergillus niger*, *Candida albicans*, for 48 h of incubation at temperature 28 ºC. The concentration of the standard drug griseofulvin was used



Scheme-I

TABLE-1
PHYSICO-CHEMICAL DATA OF SOME N-PHENYL SUBSTITUTED
2-(-5-(PYRIDINE-4-YL)-1,3,4-OXADIAZOLE-2-YL THIO)ACETAMIDES

Compound	Ar	m.f. (m.w.)	m.p. (ºC) and yield (%)	Mass	Key IR bands (ν_{max}, cm^{-1})	^1H NMR
R_1	*p*-Chloro	$C_{15}H_{11}N_4O_2SCl$ (332.810)	161	346 (M^+-1), 78 (base peak), 318, 272, 240, 220, 205, 189, 160, 127, 119	1668 (CO), 1573 (CONH), 1404 (SCH_2), 830 (P-sub. Ar. ring)	4.05 (2H, CH_2), 7.7 (2H, Ar), 7.6 (2H, Ar.), 7.23 (1H, amide), 8.75 (2H, pyridine), 7.99 (2H, pyridine).
R_2	*p*-Methoxy	$C_{16}H_{14}N_4O_3S$ (344.379)	170	342 (M^+ - 1), 78 (base peak), 268, 236, 193, 147, 134, 123, 106	1722 (CO), 1562 (CONH), 1420 (SCH_2), 837 (P-sub. Ar ring)	3.81 (3h, methyl OCH_3), 4.05 (2H, CH_2), 6.97 (2H, Ar), 7.23 (1H, amide), 7.51 (2H, Ar), 7.99 (2H, pyridine) 2H, pyridine), 8.75 (2H, pyridine).
R_3	*m*-Nitro	$C_{15}H_{13}N_5O_4S$ (359.303)	187	357 (M^+ - 1), 106 (base peak), 340, 327, 283, 238, 235, 220	1697 (CO), 1564 (CONH), 1411 (SCH_2)	4.05 (2H, CH_2), 7.69 (1H, Ar), 8 (2H, Ar), 7.23 (1H, amide), 8.74 (1H, Ar), 7.99 (2H, pyridine), 8.75 (2H, pyridine).
R_4	Phenyl	$C_{15}H_{14}N_4O_2S$ (314.355)	186	312 (M^+ - 1), 51 (base peak), 284, 238, 206, 189, 160, 124	1683 (CO), 1554 (CONH), 1410 (SCH_2)	4.05 (2H, CH_2), 7.61 (2H, Ar), 7.43 (2H, Ar), 7.23 (1H, amide), 8.75 (2H, pyridine), 7.99 (2H, pyridine).
R_5	2,4-Dinitro	$C_{15}H_{12}N_6O_6S$ (404.351)	176	183 (M^+ - 1), 52 (base peak), 167, 153, 137, 120, 107, 91	1631 (CO), 1585 (CONH), 1423 (SCH_2) 837 (P-sub. Ar ring)	4.05 (2H, CH_2), 8.07-8.77 (3H, Ar), 7.23 (1H, amide), 8.75 (2H, pyridine), 7.99 (2H, pyridine).
R_6	*o*-Chloro	$C_{15}H_{13}N_4O_2SCl$ (348.751)	186	–	1743 (CO), 1531 (CONH), 1411 (SCH_2)	4.05 (2H, CH_2), 7.13-8.04 (4H, Ar), 7.23 (1H, amide), 8.75 (2H, pyridine), 7.99 (2H, pyridine).
R_7	*p*-Carboxyl	$C_{16}H_{14}N_4O_4S$ (358.358)	260	–	1685 (COOH), 1668 (CO), 1537 (CONH), 1410 (SCH_2) 858 (P-sub. Ar ring)	4.05 (2H, CH_2), 7.87 (2H, Ar), 7.87 (2H, Ar), 7.23 (1H, amide), 8.75 (2H, pyridine), 7.99 (2H, pyridine), 11.0 (1H, COOH).
R_8	*m*-Methyl	$C_{16}H_{16}N_4O_2S$ (328.380)	160	–	1689 (CO), 1564 (CONH), 1411 (SCH_2)	2.3 (3H, CH_3), 4.05 (2H, CH_2), 6.97-7.54 (4H, Ar), 7.23 (1H, amide), 8.75 (2H, pyridine), 7.99 (2H, pyridine).
R_9	*m*-Nitro	$C_{15}H_{13}N_5O_4S$ (359.153)	142	–	1730 (CO), 1543 (CONH), 1435 (SCH_2)	4.05 (2H, CH_2), 7.7-8.58 (4H, Ar), 7.23 (1H, amide), 8.75 (2H, Ar), 7.99 (2H, pyridine).
R_{10}	*p*-Bromo	$C_{15}H_{13}N_4O_2SBr$ (413.259)	204	–	1664 (CO), 1545 (CONH), 1467 (SCH_2), 896 (P-sub. Ar ring)	4.05 (2H, CH_2),7.7 (2H, Ar), 7.6 (2H, Ar), 7.23 (1H, amide), 8.75 (2H, pyridine), 7.99 (2H, pyridine).
R_{11}	*m*-Chloro	$C_{15}H_{13}N_4OSCl_{12}$ (348.751)	160	–	1730 (CO), 1543 (CONH), 1435 (SCH_2)	4.05 (2H, CH_2), 7.23-8.0 (4H, Ar), 7.23 (1H, amide), 8.75 (2H, Ar), 7.99 (2H, pyridine).
R_{12}	*m*-Carboxyl	$C_{16}H_{14}N_4O_4S$ (358.358)	230	–	1757 (CO), 1685 (CO), 1525 (CONH), 1450 (SCH_2)	4.05 (2H, CH_2), 7.4-8.1 (4H, Ar), 7.23 (1H, amide), 8.75 (2H, pyridine), 7.99 (2H, pyridine), 11.0 (1H, COOH).
R_{13}	*p*-Methyl	$C_{16}H_{16}N_4O_2S$ (328.380)	174	326 (M^+ - 1), 106 (base peak), 252, 220, 193, 138, 106	1726 (CO), 1554 (CONH), 1465 (SCH_2), 823 (P-sub. Ar.ring)	2.3 (3H, CH_3), 4.05 (2H, CH_2), 7.21 (2H, Ar), 7.56 (2H, Ar), 7.23 (1H, amide), 8.75 (2H, pyridine), 7.99 (2H, pyridine).
R_{14}	*p*-Nitro	$C_{15}H_{13}N_5O_4S$ (359.153)	–	–	1691 (CO), 1548 (CONH), 1465 (SCH_2), 854 (P-sub. Ar ring)	4.05 (2H, CH_2), 7.82 (2H, Ar), 8.24 (2H, Ar), 7.23 (1H, amide), 8.75 (2H, pyridine), 7.99 (2H, pyridine).
R_{15}	2,4-Dinitro phenyl hydrazine	$C_{15}H_{13}N_7O_6S$ (–)	–	–	1676 (CO), 1512 (CONH), 1423 (SCH_2), 831 (P-sub. Ar ring)	4.05 (2H, CH_2), 7.35-9.0 (3H, Ar), 7.23 (1H, amide), 8.75 (2H, pyridine), 7.99 (2H, pyridine).
2	–	$C_7H_5N_3OS$ (179.000)	256	–	2677 (SH)	3.3 (1H, SHO, 7.99 (2H, Ar), 8.75 (2H, Ar).

TABLE-2
ANTIMICROBIAL ACTIVITY OF SOME N-PHENYL SUBSTITUTED 2-(-5-(PYRIDINE-4-YL)-1,3,4-OXADIAZOLE-2-YL THIO)ACETAMIDE

Compound	Zone of inhibition (mm)											
	B. subtillis		*S. aureus*		*K. pneumoniae*		*P. aeruginosa*		*C. albicans*		*A. niger*	
Conc.[n]	A	B	A	B	A	B	A	B	A	B	A	B
R_1	4	6	4	6	6	8	4	7	09	08	11	14
R_2	6	10	4	6	8	10	4	6	10	11	10	12
R_3	2	5	4	5	5	7	4	6	08	09	10	12
R_4	5	9	2	4	2	6	4	6	09	10	09	15
R_5	5	7	4	6	5	7	4	6	11	12	12	14
R_6	6	8	5	7	5	6	4	6	07	09	08	10
R_7	2	4	7	11	10	12	4	6	11	12	12	14
R_8	10	11	10	12	8	10	4	6	11	13	12	15
R_9	5	7	4	7	4	6	4	6	08	09	10	12
R_{10}	8	10	12	14	12	14	12	16	13	14	15	22
R_{11}	3	7	10	12	4	6	4	6	11	12	13	14
R_{12}	8	10	7	10	6	8	4	6	09	10	10	17
R_{13}	11	12	12	16	10	16	13	10	09	10	11	13
R_{14}	10	11	12	16	12	09	10	10	09	10	12	17
R_{15}	08	09	11	13	11	14	09	09	10	11	10	17
2	6	8	4	7	6	8	4	6	6	10	12	14
Norfloxacin 50 µg/0.1 mL									14 mm			
Griseofulvin 50 µg/0.1 m									13 mm			

A = 200 µg/0.1 mL B = 400 µg/0.1 mL all tests were performed in triplicate.

50 µg/0.1 mL and the test compounds at concentrations of 200 and 400 µg/0.1 mL.

Antitubercular activity[8,9]: The antitubercular screening was carried out by middle brook 7H9 agar medium against $H_{37}Rv$. Strain. middle brook 7H9 agar medium containing different derivatives, standard drug as well as control, middle brook 7H9 agar medium was inoculated with *Mycobacterium tuberculosis* of $H_{37}Rv$ strain. The inoculated bottles were incubated for 37 ºC for 4 weeks. At the end of 4 weeks they were checked for growth.

RESULTS AND DISCUSSION

All the compounds tested showed some degree of antimicrobial activity R_2, R_7, R_8, R_{10}, R_{12}, R_{14} and R_{15} showed good antibacterial activity (Table-2), R_5, R_{13}, R_4 and R_5 were found sensitive to tubercular microbial stains (Table-3). R_2 and R_{10} were found considerable antiinflammatory activity (Table-4). R_7, R_2, R_5 were found considerable analgesic activity (Table-5).

TABLE-3
ANTITUBERCULAR ACTIVITY OF SOME N-PHENYL SUBSTITUTED 2-(-5-(PYRIDINE-4-YL)-1,3,4-OXADIAZOLE-2-YL THIO)ACETAMIDE

Compounds	25 (mcg/mL)	50 (mcg/mL)	100 (mcg/mL)
R_1	R	R	R
R_2	R	R	R
R_3	R	R	R
R_4	R	R	R
R_5	S	S	R
R_6	R	R	R
R_7	R	R	R
R_8	R	R	R
R_9	R	R	R
R_{10}	R	R	R
R_{11}	R	R	R
R_{12}	R	R	R
R_{13}	R	S	S
R_{14}	R	S	S
R_{15}	R	S	S
3	R	R	R
Streptomycin	S	S	S
Isoniazide	S	S	S

R = Resistant, S = Sensitive, standards used streprtomycin 7.5 µg/mL; isoniazide 10 µg/mL.

TABLE-4
ANTIINFLAMMATORY ACTIVITY OF SOME N-PHENYL SUBSTITUTED 2-(-5-(PYRIDINE-4-YL)-1,3,4-OXADIAZOLE-2-YL THIO)ACETAMIDE

Compound	Antiinflammatory activity	
	Mean + SE	Inhibition (%)
R_1	3.833 ± 0.1667, N = 6 (01)	04.71
R_2	2.833 ± 0.1667, N = 6 (01)	29.71
R_3	3.333 ± 0.2108, N = 6 (01)	16.60
R_4	3.500 ± 0.2236, N = 6 (01)	12.75
R_5	3.167 ± 0.1667, N = 6 (01)	20.82
R_6	3.333 ± 0.2108, N = 6 (01)	16.67
R_7	3.500 ± 0.2236, N = 6 (01)	12.50
R_8	3.667 ± 0.2108, N = 6 (01)	08.32
R_9	3.333 ± 0.2108, N = 6 (01)	16.70
R_{10}	2.500 ± 0.2236, N = 6 (01)	37.50
R_{11}	3.500 ± 0.3416, N = 6 (01)	12.50
R_{12}	3.000 ± 0.2582, N = 6 (01)	25.00
R_{13}	3.167 ± 0.1667, N = 6 (01)	20.82
R_{14}	3.500 ± 0.2236, N = 6 (01)	12.5
R_{15}	3.333 ± 0.2108, N = 6	16.6
Control	4.000 ± 0.0000, N = 6	–
Ibuprofen	3.000 ± 0.2001, N = 6	40.00

TABLE-5
ANALGESIC ACTIVITY OF SOME N-PHENYL SUBSTITUTED 2-(-5-(PYRIDINE-4-YL)-1,3,4-OXADIAZOLE-2-YL THIO)ACETAMIDE

Compound	Analgesic activity				LD_{50}
	Tail immersion method		Writhing method		
	Mean ± SE	Inhibition (%)	Mean ± SE	Protection (%)	
R_1	3.833 ± 0.4014 N = 6 (90)	74.01	2.500 ± 0.3416 N = 6 (05)	42.27	3000 mg/kg body weight
R_2	2.833 ± 0.1667 N = 6 (120)	89.59	2.667 ± 0.2108 N = 6 (05)	38.45	3000 mg/kg body weight
R_3	3.833 ± 0.3073 N = 6 (60)	74.01	1.833 ± 0.3073 N = 6 (05)	57.70	3000 mg/kg body weight
R_4	3.667 ± 0.2108 N = 6 (90)	76.00	2.667 ± 0.3333 N = 6 (05)	38.40	3000 mg/kg body weight
R_5	1.833 ± 0.1667 N = 6 (120)	98.0	2.833 ± 0.3073 N = 6 (05)	34.62	3000 mg/kg body weight
R_6	2.833 ± 0.1667 N = 6 (60)	86.01	2.833 ± 0.4014 N = 6 (05)	34.61	3000 mg/kg body weight
R_7	3.667 ± 0.2108 N = 6 (60)	76.01	1.167 ± 0.4014 N = 6 (05)	73.06	3000 mg/kg body
R_8	3.667 ± 0.2108 N = 6 (90)	76.00	1.667 ± 0.2108 N = 6 (10)	53.34	3000 mg/kg body weight
R_9	3.833 ± 0.3073 N = 6 (60)	74.01	1.833 ± 0.3073 N = 6 (05)	57.69	3000 mg/kg body weight
R_{10}	3.833 ± 0.4773 N = 6 (60)	74.1	2.500 ± 0.2236 N = 6 (05)	42.30	3000 mg/kg body weight
R_{11}	3.833 ± 0.3073 N = 6 (30)	73.00	2.167 ± 0.1667 N = 6 (05)	49.98	3000 mg/kg body weight
R_{12}	3.500 ± 0.2236 N = 6 (120)	81.25	3.000 ± 0.3651 N = 6 (05)	30.76	3000 mg/kg body weight
R_{13}	5.667 ± 0.4216 N = 6 (150)	89.59	3.000 ± 0.3651 N = 6 (05)	30.76	3000 mg/kg body weight
R_{14}	3.833 ± 0.3073 N = 6 (60)	74.01	2.167 ± 0.1667 N = 6 (05)	49.98	3000 mg/kg body weight
R_{15}	3.500 ± 0.2236 N = 6 (120)	81.25	2.833 ± 0.4014 N = 6 (05)	34.61	3000 mg/kg body weight
Control	1.667 ± 0.2108 N = 6	–	4.500 ± 0.2236 N = 6	–	–
Pentazocin	1.833 ± 0.1667 N = 6	98.01	–	–	–
Diclofenac	–	–	1.333 ± 0.2108 N = 6	78.11	–

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