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SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL STUDIES OF SELENADIAZOLE AND HYDRAZONE DERIVATIVES OF 2, 6–DIPHENYL –4–PIPERIDONE

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ABSTRACT

Several 2, 6-diphenyl 4–piperidone derivatives were synthesized by Mannich reaction (condensation) of ethyl – methyl ketone, substituted aldehydes and ammonium acetate. The chemical structures were confirmed by means of elemental analysis, IR, and ^{1}H NMR spectral data. The synthesized compounds were evaluated for antibacterial, antifungal, acute toxicity and local anaesthetic activity at various concentrations. Among the compounds examined selenadiazole derivatives of piperidone were found to have good antibacterial and antifungal activity. The hydrazone derivatives exhibited local anaesthetic activity but selenadiazole derivatives were completely devoid of local anaesthetic activity.

Key words: 2, 6-Diphenyl-4-piperidone, Selenadiazole, Hydrazone, Acute oral toxicity, Local anaesthetic, Antimicrobial.

INTRODUCTION

In the present study, a series of 2, 6–diphenyl–4-piperidone were synthesized by condensation of ethyl methyl ketone, aromatic aldehyde and ammonium acetate by Mannich reaction[1]. Semicarbazone derivatives of 2, 6-diphenyl–4-piperidones and 2, 6-diphenyl–3-methyl–4-piperidones were synthesized by the reaction of piperidone derivatives with semicarbazide hydrochloride[2-3]. Then, these were converted into the corresponding selenadiazole compounds. The presence of ketone group in these base

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compounds was responsible for the development of annelated selenadiazole systems. 2-Substituted benzimidazoles have been synthesized by the condensation of o-phenylenediamine hydrochloride with chloroacetic acid and acetic acid[4]. Piperidin-4-one derivatives of 2, 6-diphenyl–4-piperidones and 2, 6-diphenyl–3-methyl–4-piperidones were synthesized by the reaction of 2-substituted benzimidazoles derivatives. From this, the corresponding hydrazone derivatives were synthesized. Therefore, it is envisaged that a series of 2, 6–diphenyl-piperidones and their corresponding selenadiazole and hydrazone derivatives would result in compounds of potent biological activities. These compounds were screened for their acute oral toxicity, local anaesthetic, antibacterial and antifungal activity. Among the compounds examined, selenadiazole derivatives of piperidone were found to have good antibacterial and antifungal activity. The hydrazone derivatives exhibit local anaesthetic activity but selenadiazole derivatives were completely devoid of local anaesthetic activity.

EXPERIMENTAL

All the melting points are uncorrected and were taken in open capillaries on a Gallenkamp apparatus. Majority of the reagents and chemicals procured were of AR quality and were used as received. Infrared spectra were recorded on Bruker IFS 66 V FT-IR Infrared spectrophotometer was recorded using KBr pellets. ^{1}H NMR spectra was recorded on EM-390 NMR spectrophotometer. Elemental analyses C, H, N was performed in Heraeus CHN rapid analyzer. The solvents and reagents used for the syntheses were purified by the standard methods. Purity test for the compounds were performed by TLC using glass plates coated with silica gel of 0.25 mm thickness. Spots were visualized using iodine chamber and UV light chamber.

Synthesis of compounds

The reaction scheme for the synthesis of Compounds **(3) – (11)** are shown in **Scheme 1**. The yield, physical and spectral data are shown in Table 1.

Table 1. Yield, physical and spectral data of the prepared compounds.

Comp.	Formula*	M.P (°C)	Yield (%)	Spectral data
(3)	$C_{18}H_{18}N_4O$	89	60	-

Cont....

Comp.	Formula*	M.P (°C)	Yield (%)	Spectral data
(4)	$C_{19}H_{20}N_4O$	91	73	-
(5)	$C_{17}H_{15}N_3Se$	105	60	IR (KBr, cm^{-1}): 3559 (NH); 3162 (C=Se–N stretching); 3010 (=C-H); 2900 (C-H); 2348 (N=N); 1511 (C=C); 1341 (C-N); 824 (=C-H); 662 (N-H); 516 (>C=C<). ^{1}H NMR (DMSO, δ ppm): 9.78 (m, 1H, N-H), 6.95 – 7.01 (m, 10H, Ar-H), 3.75 -3.60 (m, 2H), 2.65- 2.58 (m, 1H, methine proton at C_4, 0.85 (d, 3H, CH_3).
(6)	$C_{18}H_{17}N_3Se$	92	65	IR (KBr, cm^{-1}): 3455 (N-H); 2904 (C=Se–N stretching); 3037 (=C-H); 2868 (C-H); 2790 ^{1}(N=N); 1509 (C=C); 1304 (C-N); 783 (=C-H); 662 (N-H); 548 (>C=C<). ^{1}H NMR (DMSO, δ ppm): 9.74 (m, 1H, N-H), 6.94- 7.35 (m, 10H, Ar-H), 3.49 - 3.39 (m, 2H, methine protons at C_5 and C_7,), 2.72 - 2.65 (m, 2H, methylene protons at C_4).
(7)	$C_8H_7ClN_2$	166	67	-
(8)	$C_{25}H_{23}N_3O$	135	63	-
(9)	$C_{26}H_{25}N_3O$	131	71	-
(10)	$C_{25}H_{25}N_5$	128	62	IR (KBr, cm^{-1}): 3059 and 3027 (=C-H); 2949, 2871 and 2801 (C-H); 1660 and 1620 (N-H hydrazone); 1557 (N-H piperidine ring); 1304 (C=N); 1600, 1492 and 1450 (C=C); 1304 (C-N); 500 (>C=C<). ^{1}H NMR (DMSO, δ ppm): 7.45 -6.91 δ (m, 17H, Ar-H and N-H and NH_2), 4.76 δ (s, 2H, >N-CH_2), 3.45δ (t, 1H, axial proton at C_2 and C_6), 2.79δ - 2.50δ (d, 4H, methylene protons at C_3 and C_5).

Comp.	Formula*	M.P (°C)	Yield (%)	Spectral data
(11)	$C_{26}H_{27}N_5$	101	64	IR (KBr, cm^{-1}): 3060 and 3027 (=C-H); 2969, 2929, 2869 and 2790 (C-H stretching of methyl, methylene and methane groups); 1680 and 1623 (N-H hydrazone) , 1557 (N-H); 1305 C=N stretching of the benzimidazole ring); 1603, 1509 and 1453 (C=C); 1308 (C-N); 541 (>C=C<). ^{1}H NMR (DMSO, δ ppm): 7.47- 6.95 (m, 17H, Ar-H and N-H andNH_2), 4.75 (s, 2H, >N-CH_2), 3.48 (d, 1H, axial proton at C_6), 3.39 (t, 2H, axial proton at C_2), 2.80 - 2.51 (d, 2H , methylene protons at C_3) , 0.80 - 0.82 (d, 3H, methyl protons at C_3).

***** Elemental analyses for C, H, N are within ± 0.4% of the theoretical values.

Piperidin- 4 –semi carbazones: (3) and (4)

A mixture of semi carbazide hydrochloride (1.1 g, 0.01 mol) and piperidin–4-ones (0.01 mol) in ethanol (30 mL) was refluxed for 3 hours with continuous stirring. Then the contents were cooled. The product obtained was filtered, washed with water, vaccum dried and recrystallised from absolute ethanol.

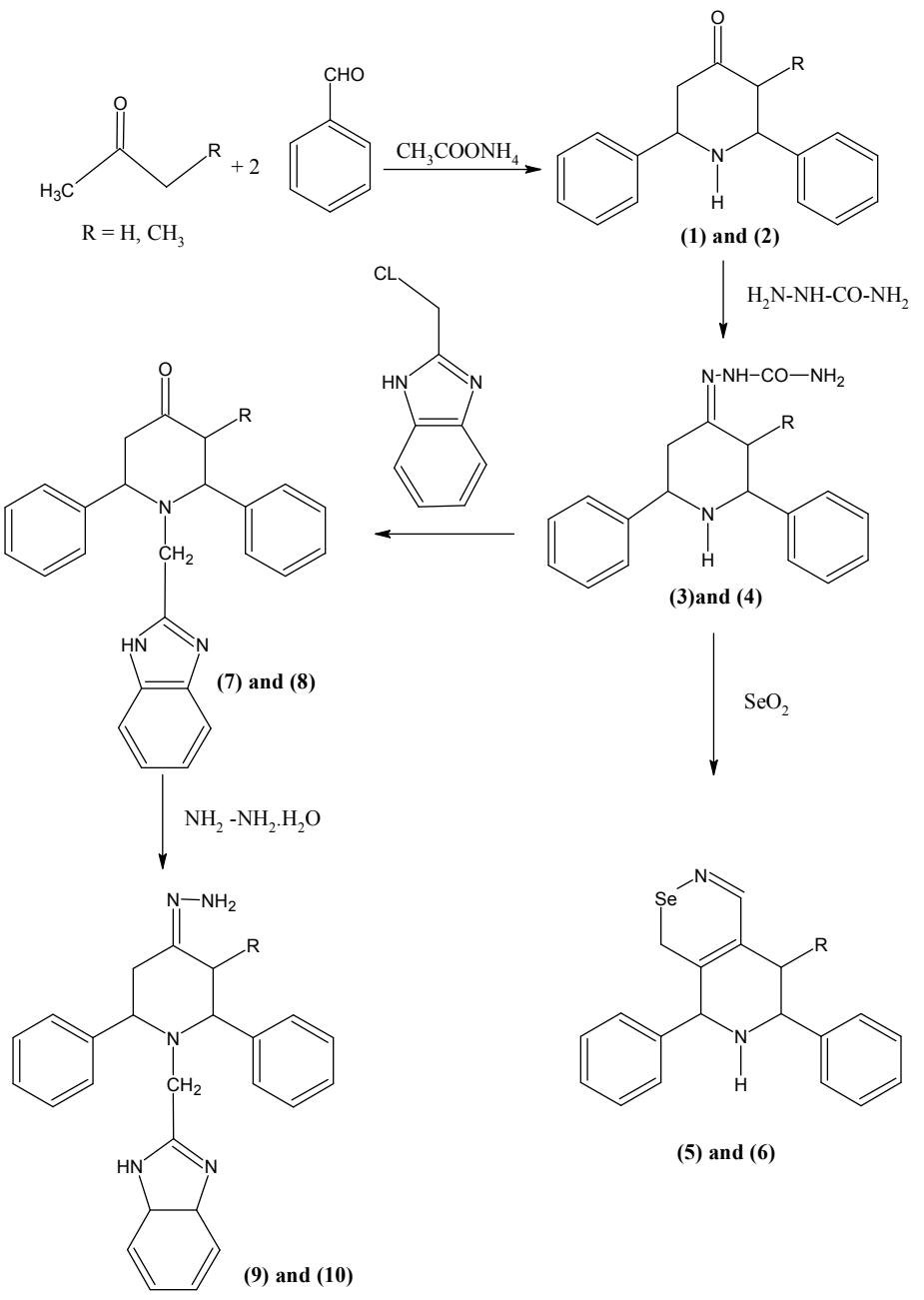
4- Piperidone[3, 4-d]- 1, 2, 3 – selenadiazole: (5) and (6)

A mixture of semicarbazone derivatives (2 g) in dioxane (5 mL) was added to an aqueous solution of selenium dioxide (0.5 g in 0.8 mL water) with stirring at room temperature for 3 hours. The product obtained was filtered, dried and recrystallised from ethanol.

2-Chloromethyl benzimidazole (7)

A mixture of o-phenylenediamine (10.8 g, 0.1 mole), chloroacetic acid (14.2 g, 0.15 mole) and 4N acetic acid (100 mL) was heated under reflux for 45 minutes. The mixture was allowed to stand overnight. It was diluted with 200 mL of water, cooled and carefully neutralized with 6N ammonium hydroxide solution. The solution was kept cold

during the neutralization and stirred well. The product was filtered, washed well with cold water, dried and recrystallised from dioxane.



Scheme 1: The reaction scheme for the synthesis of compounds

N-(Benzimidazol- 2-yl methyl- piperidin- 4- ones: (8) and (9)

A solution of piperidin - 4 – ones (0.02 mole) in 100 mL of ether containing 20 mL of absolute ethanol, was added to 2-chloromethyl benzimidazole (1.67g, 0.01 mole) in small portions, keeping the temperature below 15 ºC. The mixture was heated under reflux for four hours and then allowed to stand overnight at room temperature. Dry ether (100 mL) was added, The reaction flask was placed in an ice bath for two hours and the precipitated hydrochloride was removed. The filtrate was washed with small amount of water, dried over anhydrous sodium sulphate and then evaporated to dryness. The residue was recrystallised from ethanol.

N-(Benzimidazol-2-yl methyl) -2, 6 - diphenyl piperidin- 4- hydrazone: (10) and (11)

A mixture of N-(benzimidazol – 2-yl methyl) -2, 6 – diphenyl piperidin – 4 – ones (0.01 mole) and hydrazine hydrate (0.5 g, 0.01 mole) was dissolved in ethanol (30 mL) and it was refluxed on a steam bath for 3 hours with continuous stirring. The contents were cooled and poured into crushed ice. The precipitate obtained was filtered, washed with water, vaccum dried and recrystallised from absolute ethanol.

Pharmacology

Acute toxic activity

Acute oral toxicity test was performed as per OECE-423 guidelines (Acute toxicity class method). Wistar albino technique was used for the study. The animals were kept on fasting for 3-4 hours only with water, after which the test compounds (suspended in olive oil) were administered orally at the dose level of 5 mg/kg by intragastric tube and were observed for 3 days with mortality observed in any one animal. The same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 200, 500, 1000 and 2000 mg/kg.

Local anaesthetic activity

The local anaesthetic activity was determined by nerve block anaesthesia in frogs (n = 6). The animal was decerebrated and the upper part of the spinal cord was destroyed with a pithing needle. The abdomen was cut open and all the abdominal organs were removed and a pouch (sac) was made to expose the spinal nerves. The animal was fixed on a frog board with two of its hind legs hanging free from the board. The right leg was immersed in a beaker containing 0.1 N hydrochloric acid and the time taken for absence of

withdrawal was noted. The same procedure was repeated for the left leg also. The sac was filled with 2 mL of the test compounds at 0.5 and 1, 2 % w/v (suspended in 1% carboxymethyl cellulose) and the absence of withdrawal reflex was observed at the interval of 30 seconds. The leg was washed with normal saline between exposures to acid. Lignocaine (0.5 and 1, 2% w/v) was taken as the standard drug for comparison and the data are presented in Table 2

Table 2. Local anesthetic activity of the compounds by nerve block anaesthesia

Concentration (Percentage)	Mean absence of foot withdrawal reflex (s)				
	Lignocaine	Compound (5)	Compound (6)	Compound (10)	Compound (11)
0.5	82.50	Nil	Nil	135	195
1.0	75	Nil	Nil	112.5	150
2.0	60	Nil	Nil	82.5	135

Antibacterial activity

The test was performed according to the well diffusion method. 20 mL of the nutrient agar medium was poured into the sterile petri dishes. To the solidified plates, wells of 10 mm diameter were made using a sterile cork borer. The 24 hours subcultured bacteria were inoculated in the nutrient broth medium. After inoculating, the compounds were dissolved separately with the DMSO solvent and poured in to the wells with varying concentrations ranging from 62.5 µg/100 µL, 125 µg/100 µL, 250 µg/100 µL, and 500 µg/100 µL using a micropipette. The plates were left over for 25 hours at 37 ^{o}C. The antibiotic streptomycin was used as a standard for comparative study.

Antifungal activity

The test was performed according to the poison plate method. All the compounds were dissolved separately in the DMSO solvent for screening of their antifungal activity. The compounds were poured into the sterile Petri dishes at varying concentrations ranging from 62.5 µg/100 µL, 125 µg/100 µL, 250 µg/100 µL, and 500 µg/100 µL using micropipette. Then 20 mL of the sterilized sabourads agar medium was poured into each petri dish. After solidification of the medium, the fungal mycelia of 8 mm diameter was plunged from the fresh culture plate and inoculated into the center of the plate. Instead of the compound, the solvent DMSO was used as a standard for comparative study. The plate with the solvent alone and the mycelia was kept as the control. The plates were incubated at room temperature and after 21 days, the growth of the mycelia was measured.

RESULTS AND DISCUSSION

Compounds (**5)** and **(6)** produced mortality at 2000 mg/kg and they were considered as class-4 compounds. Compounds **(10)** and **(11)** did not cause mortality up to 2000 mg/kg and were considered as safe. Compounds **(10)** and **(11)** also exhibited significant local anaesthetic activity. The predominant and stable configuration of 2, 6-diaryl 3-substituted –piperidones is chair form with the substituents in the equatorial positions. The local anaesthetic activity of compounds may be attributed to this favourable configuration. The introduction of selenadiazole substitution probably leads to unfavourable configuration of the molecules resulting in compounds completely devoid of local anaesthetic activity.The results of the antimicrobial screening for the compounds **5**, **6**, **10** and **11** are given in the Tables 3 and 4. It was observed that when the 4-keto functionality was condensed to form selenadiazole [compound **(5)** and **(6)]** and hydrazone derivatives [compound **(10)** and **(11**)], the resulting compounds exhibit good antibacterial activity. The results of the antifungal screening for the compounds **(5)**, (**6)**, (**10)** and (**11)** are given in Table 5 and 6. From these data it is observed that the compounds **(5)** and **(6)** show more activity than compounds **(10)** and **(11)** against all dermatophytes. The increased activity against these dermatophytes by compounds **(5)** and **(6)** is attributed to the presence of selenium moiety in these compounds. In addition, the inhibition effect increases with the increase in concentration of these compounds.

Table 3. Anti bacterial activities of the compounds 5,7–diphenyl-4- piperidone[3,4-d]- 1,2,3 - selenadiazole (5) and 3-methyl-5,7–diphenyl-4-piperidone[3,4-d]-1,2,3–selenadiazole (6)

Human pathogens	Zone of inhibition of the compounds in 100 µL of DMSO against the human pathogens (mm)									
	Compound (5)					Compound (6)				
	0.0 µg	62.5 µg	125 µg	250 µg	500 µg	0.0 µg	62.5 µg	125 µg	250 µg	500 µg
Staphylococcus aureus	-	-	15.0	19.0	22.5	-	-	14.5	19.0	22.0
Bacillus subtilis	-	-	-	-	15.0	-	-	-	-	16.0
Pseudomonas aeruginosa	-	14.0	18.4	22.0	25.8	-	15.3	18.0	21.0	24.8
Salmonella typhi	-	-	16.0	18.0	19.0	-	-	-	16.0	18.6
Salmonella paratyphi 'A'	-	17.0	21.4	25.0	28.7	-	14.5	18.4	22.0	26.0
Salmonella paratyphi 'H'	-	14.0	18.0	20.5	22.0	-	13.0	16.7	18.0	24.5
Klebsiella pneumoniae	-	-	-	13.0	16.0	-		13.5	15.8	18.0
Proteus mirabilis	-	-	-	-	14.0	-	-	12.0	14.8	16.2
Proteus vulgaris	-	-	-	14.0	19.0	-	-	-	15.0	18.0
Escherichia coli	-	-	13.0	17.5	21.0	-	12.0	15.7	18.0	21.4

Table 4. Antibacterial activity of the compounds N-(benzimidazol-2-yl methyl) -2,6 - diphenyl piperidin- 4- hydrazone (9) and N-(benzimidazol- 2-yl methyl) -3 - methyl -2,6 - diphenyl piperidin4- hydrazone (10)

Human pathogens	Zone of inhibition of the compounds in 100 µL of DMSO against the Human pathogens (mm)									
	Compound (5)					Compound (6)				
	0.0 µg	62.5 µg	125 µg	250 µg	500 µg	0.0 µg	62.5 µg	125 µg	250 µg	500 µg
Staphylococcus aureus	-	-	-	12.0	16.0	-	-	-	12.0	16.0
Bacillus subtilis	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	-	17.0	23.6	27.0	29.6	-	15.0	18.0	22.8	26.0
Salmonella typhi	-	-	15.0	18.0	22.4	-	18.0	20.4	25.3	28.0
Salmonella paratyphi 'A'	-	18.0	22.5	26.0	29.5	-	14.0	17.5	21.0	25.7
Salmonella paratyphi 'H'	-	15.0	19.8	24.3	27.5	-	18.0	21.7	25.4	28.5
Klebsiella pneumoniae	-	-	12.0	18.0	18.7	-	-	-	13.5	17.6
Proteus mirabilis	-	-	-	-	-	-	-	-	-	-
Proteus vulgaris	-	-	-	-	-	-	-	-	-	-
Escherichia coli	-	-	14.0	17.8	21.0	-	-	15.0	15.0	17.0

Table 5. Anti fungal activities of the compounds 5,7–diphenyl-4- piperidone[3,4-d]- 1,2,3 - selenadiazole (5) and 3-methyl-5,7–diphenyl-4-piperidone[3,4-d]-1,2,3–selenadiazole (6)

Human pathogens	Zone of inhibition of the compounds in 100 µL of DMSO against the human pathogens (mm)									
	Compound (5)					Compound (6)				
	0.0 µg	62.5 µg	125 µg	250 µg	500 µg	0.0 µg	62.5 µg	125 µg	250 µg	500 µg
Microsporum gypseum	-	18.0	16.0	13.0	10.0	-	16.0	14.0	12.0	10.0
Microsporum nanum	-	34.0	15.0	13.0	11.0	-	32.0	15.0	13.0	11.0
Trichophyton mentagrophytes	-	40.0	23.0	17.0	14.0	-	38.0	16.0	14.0	12.0
Trichophyton rubrum	-	29.0	16.0	14.0	12.0	-	29.0	26.0	14.0	12.0

Table 6. Anti fungal activities of the compounds N-(benzimidazol-2-yl methyl) -2,6 - diphenyl piperidin- 4- hydrazone. (9) and N-(benzimidazol- 2-yl methyl) -3 - methyl -2,6 - diphenyl piperidin4- hydrazone (10)

Human pathogens	Zone of inhibition of the compounds in 100 µL of DMSO against the human pathogens (mm)									
	Compound (9)					Compound (10)				
	0.0 µg	62.5 µg	125 µg	250 µg	500 µg	0.0 µg	62.5 µg	125 µg	250 µg	500 µg
Microsporum gypseum	-	40.0	25.0	21.0	17.0	-	35.0	16.0	14.0	12.0
Microsporum nanum	-	37.0	15.0	12.0	10.0	-	34.0	16.0	14.0	13.0
Trichophyton mentagrophytes	-	38.0	18.0	15.0	13.0	-	37.0	16.0	14.0	11.0
Trichophyton rubrum	-	16.0	13.0	11.0	10.0	-	17.0	13.0	12.0	10.0

REFERENCES

1. S. Thennarasu and P. T. Perumal, Molecules, **7**, 487 (2002).
2. Krishnakumar and M. Krishnapillai, Indian J. Chem., **438**, 1088-1091 (1992).
3. Suroor Ahmad Khan and Asgar Mahmood Nandan, J. Heterocycl. Chem., 7, (1997).
4. H. O. J Collier, and Huskinson F. Patricia Ciba Foundation Symposium, Chem. and Biol. Purines 146-50, (1957).
5. D. Bhaskar Reddy, V. Padmvathi, A. Padmaja, M. V. Ramana Reddy, A. Somasekhar Reddy, A. Balaiah and T. V. Ramana Reddy, The Fifth International Electronic Conference on Synthetic Organic Chemistry (ESCOC-5), (2001) pp. 1-30.

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