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Microwave-Assisted Synthesis of Some Novel Hydrazono-4-Oxothiazolidines as Possible in Vitro Antimicrobial Activity

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Abstract:
A novel series of Thiazolidin-4-one derivatives were prepared from 2,6-diaryl-3-methyl-piperidin-4-one by microwave irradiation method. All the prepared compounds were characterised by their spectral analysis such as IR, ^{1}H NMR, ^{13}C NMR, Mass spectroscopy and C,H,N analysis. Also evaluated for their in vitro antimicrobial activity against three Gram negative strains (Pseudomonas aeruginos, Escherichia coli, Klebsiella pneumoniae), three Gram positive strains (Staphylococcus aureus, Streptococcus faecalis, Bacillus cereus) and fungal strains (Aspergillus flavus, Candida albicans, Penicllium sps, Aspergillus niger, Mucor sps and Rhizopus sps). The results revealed that all synthesized compounds have a significant biological activity against the tested micro-organisms.

Keywords*: 3-methyl-2,6-diaryl-piperidin-4-thiosemicarbazone, Diethylacetelenedicarboxylate (DEAD), thiazolidin-4-one, microwave, Montmorillonite K-10, antimicrobial activities*

1. Introduction

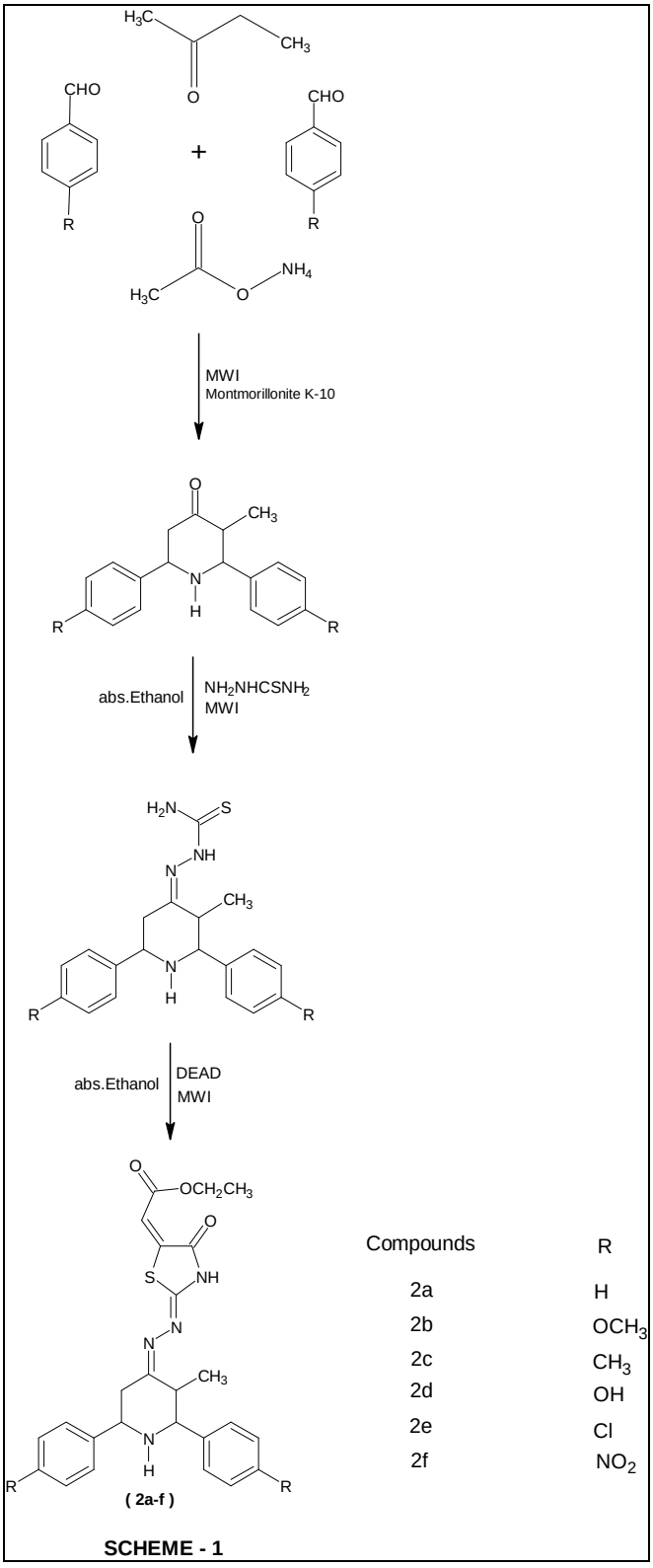
Microbial resistance to antimicrobial agents is of grave concern in the medical community. Hence, the development of novel, potent and unique antimicrobial agents is the pre-eminent way to overcome microbial resistance and develop effective therapies.

Thiazolidin-4-one derivatives, an important group of heterocyclic compounds, have been the subject of extensive study in the recent past. The development of thiazolidin-4-one chemistry has been largely associated with wide scale of applications in medicine and agriculture. Thiazolidin-4-one derivatives are known to possess anti-bacterial [1], anti-fungal [2], anti-viral [3], antischistosomal activity [4], anti-inflammatory [5], anti-HIV [6], anti-cancer [7], anti-tuberculosis [8,9], anti-malarial [10] and cytotoxic activities [11].

Piperidin-4-one heterocycles play an important role in the field of medicinal chemistry. Several 2,6-disubstituted piperidin-4-one derivatives of this class have been found to possess useful biological activities such as antibacterial and antifungal activities [12-20]. The heterocyclic compounds carrying piperidine skeleton are attractive targets in organic synthesis owing to the pharmacological activities. When one biologically active molecule is linked to another, the resultant molecule generally has increased potency. Hence in the present study the two pharmacopores i.e., substituted piperidin-4-one and 4-thiazolidinone derivatives are fused to obtain highly potent, more specific and less toxic antimicrobial agent. All above biological activities of 2,6-disubstituted piperidin-4-ones and thiazolidin-4-one derivatives aroused our attention and promoted to synthesis a new series of (E)-ethyl-2((E)-2-((Z)-(3-methyl-2,6-diarylpiperidin-4-ylidene)hydrazono)-4-oxothiazolidin-5-ylidene)acetate by microwave-irradiation method. The structure of newly synthesized compounds was confirmed by IR, ^{1}H NMR, ^{13}C NMR, Mass spectroscopy and elemental analysis. The newly synthesized compounds were screened for their antimicrobial activity against some selected bacteria and fungi.

2. Results and Discussion

The preliminary studies on spectral data and anti-microbial activity of the new series of (E)-methyl-2((E)-2-((Z)-(3-methyl-2,6-diarylpiperidin-4-ylidene)hydrazono)-4-oxothiazolidin-5-ylidene)acetate have generated some interesting data.



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2.1. Experimental Section

All the chemicals used were obtained from Sigma Aldrich, while the reagents and solvents were of analytical grade. Heating was done in microwave oven (LG, domestic oven, 900W). The melting points were determined in open capillary tube and were uncorrected. Completion of reaction and purity of synthesized compounds are checked on aluminium coated TLC plates 60 F_{245} (E. Merck) using Hexane : ethylacetate (6 :4 V/V) as mobile phase and visualized under ultraviolet (UV) light. Elemental analysis (% C, H, N) is carried out by a VarioEL III analyser. IR spectra of compounds have been recorded on Thermo-Nicolet FT-IR-200 spectrophotometer in KBr disc (cm^{-1}). ^{1}H NMR and ^{13}C NMR spectra are recorded on Bruker DRX (400 MHz) spectrometer

using DMSO as a solvent and TMS as an internal standard. Chemical shifts are reported in parts per million (δ_{ppm}). Mass spectra of synthesized compounds were carried out using the Shimadzu LCMS 2010 spectrometer.

General Procedure for the synthesis of 3-methyl-2,6-diarylpiperidin-4-ones under Microwave method

A green synthetic approach was reported for the facile synthesis of various 3-methyl-2,6-diaryl-piperidin-4-ones via Mannich-enamine substitution reaction using Montmorillonite K-10 as catalyst. Dry ammonium acetate (0.1 mole) was mixed with Montmorillonite K-10 (100 mg) in a dry condition. Freshly distilled substituted benzaldehyde (0.2 mole) and ethylmethylketone (0.1 mole) was added and the reaction mixture was placed in a microwave oven, covered with a glass funnel. The reaction mixture was irradiated with microwaves at different microwave intensities until the solution turned yellow and left at room temperature overnight. To monitor the progress of reaction, a TLC was run using Hexane : Ethylacetate (6:4) solvent system. The reaction mixture was dissolved in dry ether (10ml), treated with aqueous HCl (20ml, 1:1 (V/V)). The hydrochloride salt of the piperidin-4-one was filtered and washed with dry ether. The base was liberated from an alcoholic solution of the hydrochloride by adding a slight excess of aqueous NH_3 and diluted with water at 0^oC. The piperidin-4-one were recrystallized from abs.CH_3CH_2OH.

General procedure for the synthesis of 3-methyl-2,6-diarylpiperidin-4-thiosemicarbazone

To the mixture of 3-alkyl-2,6-diaryl-piperidin-4-one (0.05 mole) in 10ml abs. CH_3CH_2OH, few drops of con.HCl were added. Thereafter thiosemicarbazide (previously dissolved in 10ml abs.ethanol) solution (0.05 mole) was added drop-wise with constant stirring. The mixture was subject to microwave irradiation under microwave at 160W for 5 minutes. A beaker containing water was also kept in the oven to serve as a heat sink. To monitor the progress of reaction, a TLC was run to confirm the completion of reaction. After completion of reaction, the solid product was filtered off and recrystallized from abs. CH_3CH_2OH.

General procedure for the synthesis of (E)-ethyl-2((E)-2-((Z)-(3-methyl-2,6-diarylpiperidin-4-ylidene)hydrazono)-4-oxothiazolidin-5-ylidene)acetate (2a-f)

To a mixture of 3-methyl-2,6-diarylpiperidin-4-thiosemicarbazone (0.01 mole) in abs.CH_3CH_2OH (20 ml), the solution of diethylacetelenedicarboxylate (DEAD) (0.01 mole) is added in small portion. The mixture was subject to microwave irradiation under microwave at 160W for 6 min. To monitor the progress of reaction, a TLC was run to confirm the completion of reaction. After cooling, the reaction mixture to ambient temperature. The resulting yellow solid was filtered, washed with abs. CH_3CH_2OH, a yellow solid was separated.

2.1.1. Characterization data of the synthesized compounds

Characterization data of (E)-ethyl-2((E)-2-((Z)-(3-methyl-2,6-diphenylpiperidin-4-ylidene)hydrazono)-4-oxothiazolidin-5-ylidene)acetate (2a)

Yield : 81% M.p., 201^oC. IR(KBr): 1719 cm^{-1} (C=O stretching of ester), 1634 cm^{-1} (C=O stretching of thiazole ring), 1589 cm^{-1} (-C=N-), ^1H NMR (DMSO, 200 MHz): δ 2.16 (s, 1H, NH of piperidine ring), δ 6.41 (s, 1H, **H**C-CO-OCH_2CH_3)**,** δ 7.21-7.43 (m,10H, Ar-H of two phenyl ring), δ 8.37 (s, 1H, NH of thiazole ring). ^{13}C NMR (DMSO, 200 MHz): δ 124.18-128.63 ppm (Ar-C, 12C of two phenyl ring), δ 164.34 ppm (-CO-OCH_2CH_3, 1C of carbonyl carbon of ester), δ 167.38 ppm (-NH-C=O, 1C carbonyl carbon of thiazolidinone). LC-MS: m/z 463.7 (M+1), Anal. Calcd. (%) for $C_{25}H_{26}N_4O_3S$: C, 64.91; H, 5.67; N, 12.11; Found: C, 63.84; H, 5.38; N, 12.24.

Characterization data of (E)-ethyl-2((E)-2-((Z)-(3-methyl-2,6-dianisylpiperidin-4-ylidene)hydrazono)-4-oxothiazolidin-5-ylidene)acetate (2b)

Yield : 84% M.p., 209^oC. IR(KBr): 1728 cm^{-1} (C=O stretching of ester), 1642 cm^{-1} (C=O stretching of thiazole ring), 1597 cm^{-1} (-C=N-), ^1H NMR (DMSO, 200 MHz): δ 2.11 (s, 1H, NH of piperidine ring), δ 3.62 (s, 3H, OCH3 of anisyl), δ 6.36 (s, 1H, **H**C-CO-OCH_2CH_3)**,** δ 6.84-7.13 (m, 8H, Ar-H of two phenyl ring), δ 8.33 (s, 1H, NH of thiazole ring). ^{13}C NMR (DMSO, 200 MHz): δ 58.19 ppm (OCH_3,1C of anisyl), δ 117.13-125.65 ppm (Ar-C, 10C of two phenyl ring), δ 158.37-160.78 ppm (Ar-C,2C of Ar-**C**-OCH3), δ 166.37 ppm (-**C**O-OCH_2CH_3, 1C of carbonyl carbon of ester), δ 169.27 ppm (-NH-C=O, 1C carbonyl carbon of thiazolidinone). LC-MS: m/z 523.4 (M+1), Anal. Calcd. (%) for $C_{27}H_{30}N_4O_5S$: C, 62.05; H, 5.79; N, 10.72; Found: C, 61.76; H, 5.38; N, 10.13.

Characterization data of (E)-ethyl-2((E)-2-((Z)-(3-methyl-2,6-ditoluylpiperidin-4-ylidene)hydrazono)-4-oxothiazolidin-5-ylidene)acetate (2c)

Yield : 79% M.p., 217^oC. IR(KBr): 1739 cm^{-1} (C=O stretching of ester), 1645 cm^{-1} (C=O stretching of thiazole ring), 1604 cm^{-1} (-C=N-), ^1H NMR (DMSO, 200 MHz): δ 2.06 (s, 1H, NH of piperidine ring), δ 2.53 (s, 3H, CH_3 of toluyl), δ 6.38 (s, 1H, **H**C-CO-OCH_2CH_3)**,** δ 6.81-7.18 (m, 8H, Ar-H of two phenyl ring), δ 8.24 (s, 1H, NH of thiazole ring). ^{13}C NMR (DMSO, 200 MHz): δ 22.12 ppm (CH_3,1C of toluyl), δ 126.38-136.67 ppm (Ar-C, 12C of two phenyl ring), δ 165.12 ppm (-**C**O-OCH_2CH_3, 1C of carbonyl carbon of ester), δ 169.41 ppm (-NH-C=O, 1C carbonyl carbon of thiazolidinone). LC-MS: m/z 491.3 (M+1), Anal. Calcd. (%) for $C_{27}H_{30}N_4O_3S$: C, 66.10; H, 6.16; N, 11.42; Found: C, 67.17; H, 6.19; N, 11.53.

Characterization data of (E)-ethyl-2((E)-2-((Z)-(3-methyl-2,6-(4-hydroxy)diphenylpiperidin-4-ylidene)hydrazono)-4-oxothiazolidin-5-ylidene)acetate (2d)

Yield : 76% M.p., 197^oC. IR(KBr): 1753 cm^{-1} (C=O stretching of ester), 1641 cm^{-1} (C=O stretching of thiazole ring), 1617 cm^{-1} (-C=N-), ^1H NMR (DMSO, 200 MHz): δ 2.19 (s, 1H, NH of piperidine ring), δ 5.11 (s, 1H, OH of hydroxyl), δ 6.27 (s, 1H, **H**C-CO-OCH_2CH_3)**,** δ 6.71-7.06 (m, 8H, Ar-H of two phenyl ring), δ 8.26 (s, 1H, NH of thiazole ring). ^{13}C NMR (DMSO, 200 MHz): δ 113.83-155.43 ppm (Ar-C, 12C of two phenyl ring), δ 162.84 ppm (-**C**O-OCH_2CH_3, 1C of carbonyl carbon of ester), δ 165.03 ppm (-NH-C=O, 1C carbonyl carbon of thiazolidinone). LC-MS: m/z 495.7 (M+1), Anal. Calcd. (%) for $C_{25}H_{26}N_4O_5S$: C, 60.71; H, 5.30; N, 11.33; Found: C, 61.23; H, 5.38; N, 11.29.

Characterization data of (E)-ethyl-2((E)-2-((Z)-(3-methyl-2,6-(4-chloro)diphenylpiperidin-4-ylidene)hydrazono)-4-oxothiazolidin-5-ylidene)acetate (2e)

Yield : 82% M.p., 204^{o}C. IR(KBr): 1736 cm^{-1} (C=O stretching of ester), 1635 cm^{-1} (C=O stretching of thiazole ring), 1623 cm^{-1} (-C=N-), ^{1}H NMR (DMSO, 200 MHz): δ 2.18 (s, 1H, NH of piperidine ring), δ 6.28 (s, 1H, **H**C-CO-OCH$_2$CH$_3$)**,** δ 7.13-7.32 (m, 8H, Ar-H of two phenyl ring), δ 8.32 (s, 1H, NH of thiazole ring). ^{13}C NMR (DMSO, 200 MHz): δ 126.12-134.64 ppm (Ar-C, 12C of two phenyl ring), δ 161.24 ppm (-**C**O-OCH$_2$CH$_3$, 1C of carbonyl carbon of ester), δ 161.03 ppm (-NH-C=O, 1C carbonyl carbon of thiazolidinone). LC-MS: m/z 532.3 (M+1), Anal. Calcd. (%) for $C_{25}H_{24}Cl_2N_4O_3S$: C, 56.50; H, 4.55; N, 10.54; Found: C, 57.39; H, 4.58; N, 10.21.

Characterization data of (E)-ethyl-2((E)-2-((Z)-(3-methyl-2,6-(4-nitro)diphenylpiperidin-4-ylidene)hydrazono)-4-oxothiazolidin-5-ylidene)acetate (2f)

Yield : 83% M.p., 211^{o}C. IR(KBr): 1721 cm^{-1} (C=O stretching of ester), 1639 cm^{-1} (C=O stretching of thiazole ring), 1619 cm^{-1} (-C=N-), ^{1}H NMR (DMSO, 200 MHz): δ 2.18 (s, 1H, NH of piperidine ring), δ 6.31 (s, 1H, **H**C-CO-OCH$_2$CH$_3$)**,** δ 7.28-7.94 (m,8H, Ar-H of two phenyl ring), δ 8.01 (s, 1H, NH of thiazole ring). ^{13}C NMR (DMSO, 200 MHz): δ 121.89-148.18 ppm (Ar-C, 12C of two phenyl ring), δ 162.54 ppm (-**C**O-OCH$_2$CH$_3$, 1C of carbonyl carbon of ester), δ 164.08 ppm (-NH-C=O, 1C carbonyl carbon of thiazolidinone). LC-MS: m/z 553.5 (M+1), Anal. Calcd. (%) for $C_{25}H_{24}N_6O_7S$: C, 54.34; H, 4.38; N, 15.21; Found: C, 53.19; H, 4.67; N, 15.87.

2.2. Antibacterial activity of thiazolidin-4-one derivatives

The newly synthesized thiazolidin-4-one derivatives were investigated for their antibacterial activity against Gram negative strains (*Pseudomonas aeruginos, Escherichia coli, Klebsiella pneumoniae* and Gram positive strains (*Staphylococcus aureus, Streptococcus faecalis, Bacillus cereus)* by disc diffusion method. Results of antibacterial activity of above synthesized compounds are given in Table A. The synthesized compounds compared with the standard Ciprofloxacin and DMSO used as a control.

The compounds tested against six strains by the disc diffusion method. Among the synthesized compounds, Compound **2b** and **2c** showed high inhibition, compound **2a** and **2e** showed moderate activity in *Staphylococcus aureus.* Compounds **2b** and **2f** showed moderate activity against *Streptococcus faecalis* and other compounds with minimum inhibition. Compound **2d** showed high inhibition and compound **2b** and **2f** also showed moderate activity against *Escherichia coli.* In *Klebsiella pneumoniae*, compound **2b**, **2c**, and **2d** showed moderate inhibition.

2.3. Antifungal activity of thiazolidin-4-one derivatives

The synthesized 4-thiazolidinone derivatives were investigated for their antifungal activity against *Aspergillus flavus, Candida albicans, Penicillium sps, Aspergillus niger, Mucor sps* and *Rhizopus sps* fungal strains by disc diffusion method. Results of antifungal activity of the above synthesized compounds are given in Table B. The synthesized compounds are compared with the standard Amphotericin-B and DMSO used as a control.

All the synthesized compounds showed good antifungal activity than antibacterial activity. Compound **2a** and **2e** showed pronounced growth of inhibition against *Penicillium sps* and *Mucor sps*. Compound **2a** also showed good inhibition against *Penicillium sps, Mucor sps* and *Aspergillus niger*. Compound **2e** and **2a** showed good activity against *Aspergillus flavus* and compound **2e** and **2d** showed good inhibition against *Rhizopus sps.*

ANTIBACTERIAL ACTIVITY						
Compd	***Staphylococcus aureus***	***Streptococcus faecalis***	***Pseudomonas aeruginosa***	***Escherichia coli***	***Klebsiella pneumoniae***	***Bacillus cereus***
2a	7 mm	6 mm	4 mm	5 mm	5 mm	6 mm
2b	13 mm	8 mm	-	8 mm	8 mm	7 mm
2c	11 mm	5 mm	4 mm	5 mm	8 mm	6 mm
2d	5 mm	6 mm	6 mm	11 mm	8 mm	7 mm
2e	7 mm	6 mm	-	6 mm	5 mm	6 mm
2f	6 mm	8 mm	7 mm	8 mm	6 mm	7 mm
Ciprofloxacin	20 mm	16 mm	16 mm	16 mm	16 mm	14 mm
Control DMSO	-	-	-	-	-	-

Table A: Antibacterial activities of the newly synthesized compounds (Zone of inhibition in mm)

ANTIFUNGAL ACTIVITY						
Compd	***Aspergillusflavus***	***Candida albicans***	***Penicllium sps***	***Aspergillus niger***	***Mucor sps***	***Rhizopus sps***
2a	12 mm	4 mm	15 mm	11 mm	15 mm	12 mm
2b	7 mm	6 mm	8 mm	7 mm	12 mm	8 mm
2c	4 mm	3 mm	5 mm	4 mm	6 mm	5 mm
2d	10 mm	8 mm	10 mm	13 mm	8 mm	14 mm
2e	12 mm	9 mm	19 mm	11 mm	15 mm	14 mm
2f	13 mm	6 mm	11 mm	12 mm	11 mm	10 mm
Amphotericin-B	15 mm	12 mm	10 mm	10 mm	15 mm	11 mm
Control DMSO	-	-	-	-	-	-

Table B: Antifungal activities of the newly synthesized compounds (Zone of inhibition in mm)

3. Conclusion

The novel thiazolidin-4-one derivatives were synthesized by microwave irradiation method, reaction time and yield of the synthesized compound showed that the microwave irradiation method is more efficient. Synthesized compounds (2a-f) were screened for their antibacterial and antifungal activity against selected micro-organisms. The antibacterial and antifungal data revealed that the synthesized compound showed good to moderate antimicrobial activity.

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5. References

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