Journal of Enzyme Inhibition and Medicinal Chemistry, 2011; 26(1): 67–77

ISSN 1475-6366 print/ISSN 1475-6374 online
DOI: 10.3109/14756361003691878

informa
healthcare

RESEARCH ARTICLE

A facile microwave assisted green chemical synthesis of novel piperidino 2-thioxoimidazolidin-4-ones and their *in vitro* microbiological evaluation

V. Kanagarajan, J. Thanusu, and M. Gopalakrishnan

Synthetic Organic Chemistry Laboratory, Department of Chemistry, Annamalai University, Annamalai Nagar, Tamil Nadu, India

Abstract

A series of novel hybrid heterocyclic compounds, 3-(3-alkyl-2,6-diarylpiperin-4-ylidene)-2-thioxoimidazolidin-4-ones were synthesised and a comparative study was also carried out under microwave irradiation. The synthesised compounds were characterised by their melting points, elemental analysis, MS, FT-IR, one-dimensional NMR (1H, D_2O exchanged 1H and ^{13}C), two dimensional HOMOCOSY and NOESY spectroscopic data. All the synthesised title compounds were screened for their *in vitro* antibacterial and antifungal activity against clinically isolated strains namely *B. subtilis*, *M. luteus*, *S. typhii*, *S. paratyphii B*, *S. felxneri*, *P. vulgaris*, *A. niger*, *Mucor*, *Rhizopus* and *M. gypsuem* and the results were discussed.

Keywords: 3-(3-alkyl-2,6-diarylpiperin-4-ylidene)-2-thioxoimidazolidin-4-ones, $NaHSO_4.SiO_2$, microwave irradiation, antibacterial activity, antifungal activity

Introduction

Sulphur analogs of hydantoins with one or both carbonyl groups replaced by thiocarbonyl groups are called as thiohydantoins. There has been much interest in the synthesis and properties of 2-thiohydantoin derivatives used as synthetic intermediates with a wide range of applications [1,2] as hypolipidaemic, anticarcinogenic, antimutagenic, antithyroidal, antiviral (e.g. against herpes simplex virus, HSV, human immunodeficiency virus (HIV), tuberculosis, antimicrobial (antifungal and antibacterial), anti-ulcer and anti-inflammatory agents, as well as pesticides. Additionally, 2-thiohydantoins have been used as reference standards for the development of C-terminal protein sequencing, as reagents for the development of dyes and in textile printing, metal cation complexation and polymerisation catalysis. For these reasons, an alternative synthetic methodology is of paramount importance for the synthesis of 2-thiohydantoins.

Microwave irradiation is well known to promote the synthesis of a variety of compounds, where chemical reactions can be accelerated due to selective absorption of microwaves by polar molecules. Microwave-induced rate acceleration technology [3–5] has become a powerful tool in organic synthesis in view of the mild, clean, and convenient methodology and the enhanced selectivity of the reaction process in comparison to conventional solution reactions, and the associated ease of manipulation. The coupling of microwave irradiation together with solid supports under solvent free conditions have received considerable attention recently as it is one of the novel approaches to eco-friendly chemistry. Heterogeneous reactions [6–11] facilitated by supported reagents on various mineral oxides have received special attention in recent years.

Silica gel supported sodium hydrogen sulphate ($NaHSO_4.SiO_2$), is a non-toxic and inexpensive catalyst, which has been used for a variety of organic transformations [7, 9–11]. Baliah et al. have reviewed the importance of piperidin-4-ones as intermediates in the synthesis of several physiologically active compounds [12,13]. In

Address for Correspondence: M. Gopalakrishnan, Synthetic Organic Chemistry Laboratory, Department of Chemistry, Annamalai University, Annamalai Nagar, Tamil Nadu, India. Tel: + 91 4144 228 233; Email: profmgk@yahoo.co.in

(Received 25 September 2009; revised 18 January 2010; accepted 10 February 2010)

corollary of the interesting biological and pharmaceutical properties and synthetic utility, there is substantial interest in piperidones; this substructure containing compounds are widely present in numerous alkaloids and synthetically derived molecules of biological importance [14].

In view of our continued interest in the development of simpler and more convenient synthetic routes for achieving biologically challenging hybrid heterocyclic systems [3–11], we have reported a simple method to synthesise some piperidones based on 2-thiohydantoins namely 3-(3-alkyl-2,6-diarylpiperin-4-ylidene)-2-thioxoimidazolidin-4-ones with good yields by the reaction of 3-alkyl-2,6-diarylpiperidin-4-one thiosemicarbazones with chloroethyl acetate catalysed by $NaHSO_4.SiO_2$ under microwave irradiation in dry media and their *in vitro* antibacterial and antifungal activities against clinically isolated bacterial and fungal strains were evaluated.

Experimental

General remarks

TLC was used to assess the reactions and the purity of the products. All the reported melting points were taken in open capillaries and were uncorrected. A Biotage Initiator microwave synthesiser, Sweden a scientific microwave oven was used for the irradiation. IR spectra were recorded in KBr (pellet forms) on a Thermo Nicolet-Avatar–330 (Thermo Fisher Scientific Inc., Waltham, MA) FT-IR spectrophotometer (Bruker Biospin International, Ag, Aegeristrasse, Switzerland) and note worthy absorption values (cm^{-1}) were listed. One dimensional ^1H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz respectively on Bruker AMX 400 NMR spectrometer (Bruker Biospin International, Ag, Aegeristrasse, Switzerland) using DMSO-*d* as solvent. Two dimensional HOMOCOSY and NOESY spectra were recorded at 500 MHz on Bruker DRX 500 NMR spectrometer using DMSO-*d* as solvent. The electron spray impact (ESI) positive (+ve) mass (MS) spectra were recorded on a Bruker Daltonics LC-MS spectrometer. Satisfactory microanalysis was obtained on Carlo Erba 1106 CHN analyser (Thermo Fisher Scientific Inc., Waltham, MA).

General procedure for the synthesis of 3-alkyl-2, 6-diarylpiperidin-4-ones 1–15

A mixture of ammonium acetate (0.1 mol), the respective substituted benzaldehyde (0.2 mol) and appropriate ketone (0.1 mol) (s.d. Fine chemicals, Mumbai, India) were dissolved in 95% alcohol (80 mL) and the solution was heated on a hot plate with gentle swirling until the colour of the mixture changed to orange. The mixture was cooled and poured into diethyl ether (100 mL) and concentrated hydrochloric acid (14 mL) was added. The precipitated hydrochloride was collected by filtration and recrystallised from the ethanol-ether mixture. The hydrochloride was dispersed in acetone and concentrated aqueous ammonia added dropwise until a clear solution was obtained. The clear solution was poured into cold water and the solid precipitation was collected and recrystallised from ethanol.

General procedure for the synthesis of 3-alkyl-2, 6-diarylpiperidin-4-one thiosemicarbazones 16–30

A mixture of the respective 3-substituted-2,6-diarylpiperidin-4-ones **1**–**15** (0.01 mol) and thiosemicarbazide (0.01 mol) in ethanol (60 mL) was refluxed for 2 h on a steam bath. It was cooled and then the separated solid was filtered and washed with water. The solid was subjected to column chromatography using benzene:chloroform (1:1) as eluent to afford compounds **16**–**30**.

General procedure for the synthesis of 3-(3-alkyl-2, 6-diarylpiperin-4-ylidene)-2-thioxoimidazolidin-4-ones 31–45

To a well stirred solution of 3-alkyl-2,6-diphenylpiperidin-4-one thiosemicarbazones **31**–**45** (0.01 mmol) and anhydrous sodium acetate (0.01 mol) in 30 mL of ethanol, chloroethyl acetate (0.01 mmol) in 15 mL of ethanol was added drop wise through a funnel for about 10 min and the reaction mixture was refluxed for 4 h. After completion of the reaction, the reaction mixture was poured into ice cold water and the solid mass was collected and recrystallised twice from ethanol.

General procedure for the microwave assisted synthesis of 3-(3-alkyl-2,6-diarylpiperin-4-ylidene)-2-thioxoimidazolidin-4-ones catalysed by $NaHSO_4.SiO_2$ 31–45

A mixture containing 3-alkyl-2,6-diphenylpiperidin-4-ones **1**–**15** (0.01 mmol), thiosemicarbazide (0.01 mmol), and chloroethyl acetate (0.01 mmol) and $NaHSO_4.SiO_2$ (25 mg) was added in an alumina bath, mixed thoroughly with the aid of a glass rod (10 s) and then irradiated in a microwave oven for 2–4 min. at 320 W (monitored by TLC). After completion of the reaction, the reaction mixture was extracted with dichloromethane (3 × 5 mL). The catalyst and other solid wastes were removed by filtration. The combined organic layer was washed with water three times and then dried over anhydrous $MgSO_4$. The organic layer was concentrated *in vacuo* to furnish the crude products and recrystallised twice from ethanol.

Spectroscopic data

3-(2,6-diphenylpiperidin-4-ylideneamino)-2-thioxoimidazolidin-4-one 31

Irradiation reaction time = 3 min; IR (KBr) (cm^{-1}): 3400, 3306, 3060, 3029, 2980, 2896, 2797, 1728, 1635, 1598, 1215, 701, 758, 1041; ^1H NMR (δ ppm): 1.97–2.05 (*m*, 1H, H_{3a}), 2.37–2.41 (*dd*, 1H, H_{3e}, $J_{3e,3a}$ = 13.64 Hz, $J_{3e,2a}$ = 2.96 Hz); 2.43–2.52 (*m*, 1H, H_{5a}), 2.83 (*s*, 1H, NH of piperidine), 3.62–3.66 (*dd*, H_{5e}, $J_{5e,5a}$ = 2.96 Hz, $J_{5e,6a}$ = 13.52 Hz), 3.8 (*s*, 2H, CH_2 of imidazolidine), 3.88–3.92 (*dd*, 1H, H_{2a},

$J_{2a,3e}$ = 3.08 Hz, $J_{2a,3a}$ = 11.76 Hz), 4.15–4.19 (*dd*, 1H, H_{6a}, $J_{6a,5e}$ = 3.2 Hz, $J_{6a,5a}$ = 11.88 Hz), 7.23–7.5 (*m*, 10H, Ar-H's), 11.78 (*s*, NH of imidazolidine); In the D_2O exchanged ^1H NMR spectrum, two peaks at 2.83 ppm and 11.78 ppm which resonances due to NH of piperidine and imidazolidine respectively disappeared; ^{13}C NMR (δ ppm): 29.6 C-3, 37.3 C-5, 43.6 CH_2 of imidazolidine, 60.2 C-2, 61.1 C-6, 126.5–128.1 Ar-C's, 144, 144.1 *ipso*-C, 163.1 C=N, 167.6 C=O, 173.8 C=S.

3-(2,6-bis(4-fluorophenyl)piperidin-4-ylideneamino)-2-thioxoimidazolidin-4-one 32

Irradiation reaction time = 4 min; IR (KBr) (cm^{-1}): 3430, 3317, 3076, 3030, 2956, 2924, 2854, 1717, 1641, 1604, 1092, 835, 733, 519; ^1H NMR (δ ppm): 1.98–2.05 (*m*, 1H, H_{3a}), 2.39–2.44 (*dd*, 1H, H_{3e}, $J_{3e,3a}$ = 13.52 Hz, $J_{3e,2a}$ = 2.96 Hz); 2.44–2.49 (*m*, 1H, H_{5a}), 2.86 (*s*, 1H, NH of piperidine), 3.51–3.55 (*dd*, 1H, H_{5e}, $J_{5e,5a}$ = 2.96 Hz, $J_{5e,6a}$ = 13.4 Hz), 3.77 (*s*, 2H, CH_2 of imidazolidine), 3.85–3.89 (*dd*, 1H, H_{2a}, $J_{2a,3e}$ = 2.96 Hz, $J_{2a,3a}$ = 11.76 Hz), 4.16–4.2 (*dd*, 1H, H_{6a}, $J_{6a,5e}$ = 3.32 Hz, $J_{6a,5a}$ = 11.96 Hz), 7.18–7.37 (*m*, 8H, Ar-H's), 11.81 (*s*, 1H, NH of imidazolidine); ^{13}C NMR (δ ppm): 30.1 C-3, 38.2 C-5, 43.4 CH_2 of imidazolidine, 61.8 C-2, 62.9 C-6, 114.3–142.6 Ar-Cs, 142.9, 159.6 *ipso*-C, 162 C=N, 166.7 C=O, 173.7 C=S.

3-(2,6-bis(4-chlorophenyl)piperidin-4-ylideneamino)-2-thioxoimidazolidin-4-one 33

Irradiation reaction time = 4 min; IR (KBr) (cm^{-1}): 3400, 3309, 3065, 3032, 2978, 2923, 2852, 1724, 1628, 1595, 1195, 1014, 826, 722, 676, 634; ^1H NMR (δ ppm): 1.98–2.05 (*m*, 1H, H_{3a}), 2.38–2.42 (*dd*, 1H, H_{3e}, $J_{3e,3a}$ = 13.52 Hz, $J_{3e,2a}$ = 2.98 Hz); 2.43–2.48 (*m*, 1H, H_{5a}), 2.79 (*s*, 1H, NH of piperidine), 3.52–3.56 (*dd*, 1H, H_{5e}, $J_{5e,5a}$ = 3.08 Hz, $J_{5e,6a}$ = 13.42 Hz), 3.78 (*s*, 2H, CH_2 of imidazolidine), 3.87–3.91 (*dd*, 1H, H_{2a}, $J_{2a,3e}$ = 3.08 Hz, $J_{2a,3a}$ = 11.76 Hz), 4.15–4.19 (*dd*, 1H, H_{6a}, $J_{6a,5e}$ = 3.14 Hz, $J_{6a,5a}$ = 11.70 Hz), 7.3–7.58 (*m*, 8H, Ar-H's), 11.79 (*s*, 1H, NH of imidazolidine); ^{13}C NMR (δ ppm): 30 C-3, 38.8 C-5, 43.5 CH_2 of imidazolidine, 60.9 C-2, 61.2 C-6, 126.7–140.4 Ar-C's, 144.7, 145.3 *ipso*-C, 163.1 C=N, 166.8 C=O, 173.5 C=S.

3-(2,6-bis(4-methoxyphenyl)piperidin-4-ylideneamino)-2-thioxoimidazolidin-4-one 34

Irradiation reaction time = 3 min; IR (KBr) (cm^{-1}): 3400, 3306, 3065, 3020, 2962, 2924, 2853, 1728, 1630, 1601, 1251, 1031, 830, 749, 527; ^1H NMR (δ ppm): 1.97–2.04 (*m*, 1H, H_{3a}), 2.35–2.39 (*dd*, 1H, H_{3e}, $J_{3e,3a}$ = 13.52 Hz, $J_{3e,2a}$ = 2.96 Hz); 2.43–2.5 (*m*, 1H, H_{5a}), 2.89 (*s*, 1H, NH of piperidine), 3.48–3.53 (*dd*, 1H, H_{5e}, $J_{5e,5a}$ = 3.16 Hz, $J_{5e,6a}$ = 13.4 Hz), 3.59 (*s*, 6H, OCH_3 at the phenyl rings), 3.72 (*s*, 2H, CH_2 of imidazolidine), 3.87–3.91 (*dd*, 1H, H_{2a}, $J_{2a,3e}$ = 3.16 Hz, $J_{2a,3a}$ = 11.68 Hz), 4.13–4.17 (*dd*, 1H, H_{6a}, $J_{6a,5e}$ = 3.08 Hz, $J_{6a,5a}$ = 11.68 Hz), 7.19–7.42 (*m*, 8H, Ar-H's), 11.78 (*s*, 1H, NH of imidazolidine); ^{13}C NMR (δ ppm): 30.1 C-3, 37 C-5, 43.1 CH_2 of imidazolidine, 54.2 -OCH_3 at the phenyl rings, 60.2 C-2, 61.1 C-6, 127.3–144.0 Ar-C's, 144.1, 159.9, *ipso*-C, 162.3 C=N, 167.7 C=O, 173.8 C=S.

3-(2,6-dip-tolylpiperidin-4-ylideneamino)-2-thioxoimidazolidin-4-one 35

Irradiation reaction time = 3 min; IR (KBr) (cm^{-1}): 3403, 3308, 3063, 3018, 2960, 2921, 2850, 1727, 1631, 1600, 1249, 1023, 828, 742, 524; ^1H NMR (δ ppm): 1.97–2.05 (*m*, 1H, H_{3a}), 2.18 (*s*, 6H, CH_3 at the phenyl rings), 2.34–2.38 (*dd*, 1H, H_{3e}, $J_{3e,3a}$ = 13.5 Hz, $J_{3e,2a}$ = 2.97 Hz); 2.45–2.52 (*m*, 1H, H_{5a}), 2.84 (*s*, 1H, NH of piperidine), 3.49–3.52 (*dd*, 1H, H_{5e}, $J_{5e,5a}$ = 3.14 Hz, $J_{5e,6a}$ = 13.42 Hz), 3.76 (*s*, 2H, CH_2 of imidazolidine), 3.88–3.9 (*dd*, 1H, H_{2a}, $J_{2a,3e}$ = 3.14 Hz, $J_{2a,3a}$ = 11.66 Hz), 4.14–4.18 (*dd*, 1H, H_{6a}, $J_{6a,5e}$ = 3.07 Hz, $J_{6a,5a}$ = 11.66 Hz), 7.17–7.39 (*m*, 8H, Ar-H's), 11.76 (*s*, 1H, NH of imidazolidine); ^{13}C NMR (δ ppm): 22.2 -CH_3 at the phenyl rings, 30.3 C-3, 37.2 C-5, 43.3 CH_2 of imidazolidine, 60.1 C-2, 61 C-6, 127.1–143.9 Ar-C's, 144.1, 147.2, *ipso*-C, 162.8 C=N, 167.5 C=O, 173.5 C=S.

3-(3-methyl-2,6-diphenylpiperidin-4-ylideneamino)-2-thioxoimidazolidin-4-one 36

Irradiation reaction time = 2 min; IR (KBr) (cm^{-1}): 3420, 3311, 3063, 3031, 2978, 2933, 1713, 1634, 1600, 1232, 755, 702; ^1H NMR (δ ppm): 0.77–0.79 (*d*, 3H, CH_3 at C-3, $J_{CH3,3a}$ = 6.45 Hz), 2.05–2.11 (*m*, 1H, H_{5a}), 2.55–2.59 (*m*, 1H, H_{3a}), the signal for 1H, NH of piperidine merged with water peak, 2.69–2.79 (*m*, 1H, H_{5e}), 3.45–3.48 (*d*, 1H, H_{2a}, $J_{2a,3a}$ = 9.98 Hz), 3.54–3.58 (*dd*, 1H, H_{6a}, $J_{6a,5e}$ = 2.5 Hz, $J_{6a,5a}$ = 12.95 Hz), 3.76 (*s*, 2H, CH_2 of imidazolidine), 7.24–7.47 (*m*, 10H, Ar-H's), 11.71 (*s*, NH of imidazolidine); ^{13}C NMR (δ ppm): 11.9 -CH_3 at C-3, 32.5 C-5, 37.3 C-3, 44.4 CH_2 of imidazolidine, 60.4 C-6, 68.7 C-2, 126.5–128.1 Ar-C's, 144.9, 144, *ipso*-C, 163.1 C=N, 167.5 C=O, 173.8 C=S.

3-(2,6-bis(4-fluorophenyl)-3-methylpiperidin-4-ylideneamino)-2-thioxoimidazolidin-4-one 37

Irradiation reaction time = 3 min; IR (KBr) (cm^{-1}): 3412, 3304, 3071, 3030, 2985, 2931, 2869, 1717, 1635, 1597, 1221, 1030, 535, 836, 766; ^1H NMR (δ ppm): 0.78–0.8 (*d*, 3H, CH_3 at C-3, $J_{CH3,3a}$ = 6.58 Hz), 2.07–2.09 (*m*, 1H, H_{5a}), 2.54–2.59 (*m*, 1H, H_{3a}), the signal for 1H, NH of piperidine merged with water peak, 2.67–2.79 (*m*, 1H, H_{5e}), 3.46–3.48 (*d*, 1H, H_{2a}, $J_{2a,3a}$ = 10.61 Hz), 3.54–3.58 (*dd*, 1H, H_{6a}, $J_{6a,5e}$ = 2.46 Hz, $J_{6a,5a}$ = 12.46 Hz), 3.78 (*s*, 2H, CH_2 of imidazolidine), 7.12–7.51 (*m*, 8H, Ar-H's), 11.72 (*s*, NH of imidazolidine); ^{13}C NMR (δ ppm): 11.8 -CH_3 at C-3, 32.5 C-5, 37.2 C-3, 44.5 CH_2 of imidazolidine, 61 C-6, 67.7 C-2, 114.6–160.7 Ar-C's, 162.4, 162.5, *ipso*-C, 163.4 C=N, 169.2 C=O, 173.9 C=S.

3-(2,6-bis(4-chlorophenyl)-3-methylpiperidin-4-ylideneamino)-2-thioxoimidazolidin-4-one 38

Irradiation reaction time = 4 min; IR (KBr) (cm^{-1}): 3429, 3282, 3070, 3032, 2985, 2931, 1718, 1625, 1592, 1210, 1017, 897, 823; ^1H NMR (δ ppm): 0.77–0.79 (*d*, 3H, CH_3 at C-3, $J_{CH3,3a}$ = 6.57 Hz), 2.01–2.07 (*m*, 1H, H_{5a}), 2.53–2.57 (*m*, 1H, H_{3a}), the signal for 1H, NH of piperidine merged with water peak, 2.68–2.8 (*m*, 1H, H_{5e}), 3.46–3.48 (*d*, 1H, H_{2a}, $J_{2a,3a}$ = 10.82 Hz), 3.54–3.58 (*dd*, 1H, H_{6a}, $J_{6a,5e}$ = 2.44 Hz, $J_{6a,5a}$ = 12.45 Hz), 3.9 (*s*, 2H, CH_2 of imidazolidine),

7.37–7.49 (*m*, 8H, Ar-H's), 11.74 (*s*, NH of imidazolidine); ^{13}C NMR (δ ppm): 11.8 -CH_3 at C-3, 31.8 C-5, 36.8 C-3, 44.3 CH_2 of imidazolidine, 61 C-6, 67.7 C-2, 128-131.7 Ar-C's, 141.8, 142.8 *ipso*-C, 160.8 C=N, 166.9 C=O, 171.5 C=S.

3-(2,6-bis(4-methoxyphenyl)-3-methylpiperidin-4-ylideneamino)-2-thioxoimidazolidin-4-one 39

Irradiation reaction time = 2 min; IR (KBr) (cm^{-1}): 3426, 3286, 3080, 2981, 2927, 1722, 1627, 1594, 1200, 1025, 897, 812, 744, 527; ^{1}H NMR (δ ppm): 0.78–0.8 (*d*, 3H, CH_3 at C-3, $J_{CH3,3a}$ = 6.45 Hz), 2.04–2.08 (*m*, 1H, H_{5a}), 2.55–2.59 (*m*, 1H, H_{3a}), the signal for 1H, NH of piperidine merged with water peak, 2.69–2.79 (*m*, 1H, H_{5e}), 3.46–3.48 (*d*, 1H, H_{2a}, $J_{2a,3a}$ = 10.72 Hz), 3.54–3.58 (*dd*, 1H, H_{6a}, $J_{6a,5e}$ = 2.56 Hz, $J_{6a,5a}$ = 12.87 Hz), 3.28 (*s*, 6H, OCH_3 at the phenyl rings), 3.78 (*s*, 2H, CH_2 of imidazolidine), 6.86–7.36 (*m*, 8H, Ar-H's), 11.82 (*s*, NH of imidazolidine); ^{13}C NMR (δ ppm): 11.9 -CH_3 at C-3, 32.5 C-5, 37.3 C-3, 44.6 CH_2 of imidazolidine, 54.9 -OCH_3 at the phenyl rings, 60.5 C-6, 68.1 C-2, 127.6–158.4 Ar-C's, 160.5, 163.1 *ipso*-C, 166.9 C=N, 169.7 C=O, 173.9 C=S.

3-(3-methyl-2,6-dip-tolylpiperidin-4-ylideneamino)-2-thioxoimidazolidin-4-one 40

Irradiation reaction time = 2 min; IR (KBr) (cm^{-1}): 3441, 3312, 3229, 3060, 2971, 2931, 1727, 1634, 1607, 1247, 1031, 542, 833, 753; ^{1}H NMR (δ ppm): 0.78–0.8 (*d*, 3H, CH_3 at C-3, $J_{CH3,3a}$ = 6.38 Hz), 2.01–2.07 (*m*, 1H, H_{5a}), 2.19 (*s*, 6H, CH_3 at the phenyl rings), 2.54–2.58 (*m*, 1H, H_{3a}), the signal for 1H, NH of piperidine merged with water peak, 2.69–2.79 (*m*, 1H, H_{5e}), 3.44–3.47 (*d*, 1H, H_{2a}, $J_{2a,3a}$ = 10.85 Hz), 3.54–3.58 (*dd*, 1H, H_{6a}, $J_{6a,5e}$ = 2.55 Hz, $J_{6a,5a}$ = 12.89 Hz), 3.89 (*s*, 2H, CH_2 of imidazolidine), 7.1–7.33 (*m*, 8H, Ar-H's), 11.75 (*s*, NH of imidazolidine); ^{13}C NMR (δ ppm): 11.9 -CH_3 at C-3, 20.6 -CH_3 at the phenyl rings, 32.5 C-5, 37 C-3, 44.5 CH_2 of imidazolidine, 60.2 C-6, 68.4 C-2, 126.3–136.2 Ar-C's, 139.9, 140.9 *ipso*-C, 166.8 C=N, 169.6 C=O, 171.5 C=S.

3-(3,3-dimethyl-2,6-diphenylpiperidin-4-ylideneamino)-2-thioxoimidazolidin-4-one 41

Irradiation reaction time = 2 min; IR (KBr) (cm^{-1}): 3496, 3307, 3060, 3027, 2973, 2926, 2852, 1719, 1625, 1206, 1028, 758, 703; ^{1}H NMR (δ ppm): 0.97 (*s*, 3H, CH_3 at C-4), 1.19 (*s*, 3H, CH_3 at C-4), 2.52–2.56 (*dd*, 1H, H_{5e}, $J_{5e,5a}$ = 13.96 Hz, $J_{5e,6a}$ = 3.64 Hz), 2.75 (*s*, 1H, H_1); 3.18–3.25 (*m*, 1H, H_5a), 3.72 (*s*, 1H, H_{2a}), 3.91 (*s*, 2H, CH_2 of imidazolidine), 4.13–4.18 (*dd*, 1H, H_{6a}, $J_{6a,5e}$ = 3.64 Hz, $J_{6a,5a}$ = 14.44 Hz), 7.21–7.53 (*m*, 10H, H_{arom}), 11.8 (*s*, 1H, NH of imidazolidine); ^{13}C NMR (δ ppm): 20.4, 20.8 two CH_3 at C-4, 43.5 CH_2 of imidazolidine, 46.4 C-5, 48.8 C-3, 61 C-6, 69.8 C-2, 126.5–140.3 Arom-C's, 143.5, 144.2 *ipso* Cs, 163.1 C=N, 167.3 C=O, 173.8 C=S.

3-(2,6-bis(4-fluorophenyl)-3,3-dimethylpiperidin-4-ylideneamino)-2-thioxoimidazolidin-4-one 42

Irradiation reaction time = 3 min; IR (KBr) (cm^{-1}): 3480, 3309, 3147, 3072, 2975, 2923, 1719, 1628, 1222, 836, 702, 656, 525; ^{1}H NMR (δ ppm): 0.96 (*s*, 3H, CH_3 at C-4), 1.18 (*s*, 3H, CH_3 at C-4), 2.53–2.58 (*dd*, 1H, H_{5e}, $J_{5e,5a}$ = 13.8 Hz, $J_{5e,6a}$ = 3.6 Hz), 2.8 (*s*, 1H, H_1); 3.19–3.27 (*m*, 1H, H_5a), 3.78 (*s*, 1H, H_{2a}), 3.92 (*s*, 2H, CH_2 of imidazolidine), 4.15–4.19 (*dd*, 1H, H_{6a}, $J_{6a,5e}$ = 3.6 Hz, $J_{6a,5a}$ = 14.44 Hz), 7.3–7.59 (*m*, 8H, H_{arom}), 11.79 (*s*, 1H, NH of imidazolidine); ^{13}C NMR (δ ppm): 20.3, 20.7 two CH_3 at C-4, 43.5 CH_2 of imidazolidine, 46.4 C-5, 48.8 C-3, 61 C-6, 69.9 C-2, 113.9–140.4 Arom-C's, 160.4, 160.6 *ipso* Cs, 163.3 C=N, 166.9 C=O, 173.8 C=S.

3-(2,6-bis(4-chlorophenyl)-3,3-dimethylpiperidin-4-ylideneamino)-2-thioxoimidazolidin-4-one 43

Irradiation reaction time = 4 min; IR (KBr) (cm^{-1}): 3400, 3306, 3093, 3030, 2974, 2928, 2852, 1722, 1630, 1203, 1015, 827, 766, 523; ^{1}H NMR (δ ppm): 0.98 (*s*, 3H, CH_3 at C-4), 1.28 (*s*, 3H, CH_3 at C-4), 2.54–2.58 (*dd*, 1H, H_{5e}, $J_{5e,5a}$ = 13.92 Hz, $J_{5e,6a}$ = 3.64 Hz), 2.81 (*s*, 1H, H_1); 3.18–3.25 (*m*, 1H, H_5a), 3.77 (*s*, 1H, H_{2a}), 3.94 (*s*, 2H, CH_2 of imidazolidine), 4.16–4.21 (*dd*, 1H, H_{6a}, $J_{6a,5e}$ = 3.64 Hz, $J_{6a,5a}$ = 14.48 Hz), 7.37–7.89 (*m*, 8H, H_{arom}), 11.85 (*s*, 1H, NH of imidazolidine); ^{13}C NMR (δ ppm): 20.2, 20.8 two CH_3 at C-4, 43.4 CH_2 of imidazolidine, 46 C-5, 48.5 C-3, 61.4 C-6, 68.6 C-2, 113.4–139.2 Arom-C's, 158.8, 159.2 *ipso* Cs, 163.2 C=N, 165.9 C=O, 173.5 C=S.

3-(2,6-bis(4-methoxyphenyl)-3,3-dimethylpiperidin-4-ylideneamino)-2-thioxoimidazolidin-4-one 44

Irradiation reaction time = 3 min; IR (KBr) (cm^{-1}): 3448, 3317, 3098, 3027, 2976, 2924, 2861, 1702, 1626, 1211, 1028, 818, 747, 519; ^{1}H NMR (δ ppm): 0.98 (*s*, 3H, CH_3 at C-4), 1.16 (*s*, 3H, CH_3 at C-4), 2.54–2.58 (*dd*, 1H, H_{5e}, $J_{5e,5a}$ = 13.94 Hz, $J_{5e,6a}$ = 3.66 Hz), 2.76 (*s*, 1H, H_1); 3.19–3.25 (*m*, 1H, H_5a), 3.56 (*s*, 6H, OCH_3 at the phenyl rings),3.79 (*s*, 1H, H_{2a}), 3.91 (*s*, 2H, CH_2 of imidazolidine), 4.17–4.22 (*dd*, 1H, H_{6a}, $J_{6a,5e}$ = 3.66 Hz, $J_{6a,5a}$ = 14.5 Hz), 7.19–7.76 (*m*, 8H, H_{arom}), 11.79 (*s*, 1H, NH of imidazolidine); ^{13}C NMR (δ ppm): 20.3, 20.7 two CH_3 at C-4, 43.5 CH_2 of imidazolidine, 46.5 C-5, 48.9 C-3, 54 -OCH_3 at the phenyl rings, 61 C-6, 68.2 C-2, 126.4–141.1 Arom-C's, 141.3, 142.2 *ipso* C's, 163.1 C=N, 166.8 C=O, 173.8 C=S.

3-(3,3-dimethyl-2,6-dip-tolylpiperidin-4-ylideneamino)-2-thioxoimidazolidin-4-one 45

Irradiation reaction time = 2 min; IR (KBr) (cm^{-1}): 3450, 3318, 3093, 3022, 2974, 2925, 2864, 1705, 1628, 1209, 1023, 817, 749, 516; ^{1}H NMR (δ ppm): 0.99 (*s*, 3H, CH_3 at C-4), 1.18 (*s*, 3H, CH_3 at C-4), 2.24 (*s*, 6H, CH_3 of phenyl rings), 2.55–2.59 (*dd*, 1H, H_{5e}, $J_{5e,5a}$ = 13.96 Hz, $J_{5e,6a}$ = 3.68 Hz), 2.77 (*s*, 1H, H_1); 3.19–3.26 (*m*, 1H, H_5a), 3.8 (*s*, 1H, H_{2a}), 3.92 (*s*, 2H, CH_2 of imidazolidine), 4.18–4.23 (*dd*, 1H, H_{6a}, $J_{6a,5e}$ = 3.68 Hz, $J_{6a,5a}$ = 14.52 Hz), 7.21–7.8 (*m*, 8H, H_{arom}), 11.8 (*s*, 1H, NH of imidazolidine); ^{13}C NMR (δ ppm): 20.3, 20.7 two CH_3 at C-4, 22.7 (-CH_3 of phenyl rings), 43.5 CH_2 of imidazolidine, 46.5 C-5, 48.9 C-3, 61 C-6, 68.2 C-2, 126.4–141.1 Arom-C's, 141.3, 142.2 *ipso* Cs, 163.1 C=N, 166.8 C=O, 173.8 C=S.

Microbiology

Materials

All the clinically isolated bacterial strains namely *Bacillus subtilis*, *Micrococcus luteus*, *Salmonella typhii*, *Salmonella*

paratyphii-B, *Shigella felxneri*, *Proteus vulgaris* and fungal strains namely *Aspergillus niger*, *Mucor*, *Rhizopus* and *Microsporum gypsuem*, were obtained from the Faculty of Medicine, Annamalai University, Annamalainagar-608 002, Tamil Nadu, India.

In vitro antibacterial and antifungal activity

The MIC as µg/mL values was determined by a two-fold serial dilution method [15]. The respective test compounds **31**–**45** were dissolved in dimethyl sulphoxide (DMSO) to obtain a 1 mg mL^{-1} stock solution. Seeded broth (broth containing microbial spores) was prepared in nutrient broth (NB) from 24 h old bacterial cultures on nutrient agar (Hi-media, Mumbai) at 37 ± 1°C while fungal spores from 1 to 7 days old Sabouraud's agar (Hi-media, Mumbai) slant cultures were suspended in Sabouraud's dextrose broth (SDB). The colony forming units (CFU) of the seeded broth were determined by a plating technique and adjusted within the range of 10^4–10^5 CFU/mL. The final inoculum size was 10^5 CFU/mL for the antibacterial assay and 1.1–1.5 × 10^2 CFU/mL for the antifungal assay. The testing was performed at pH 7.4 ± 0.2 for bacteria (NB) and at pH 5.6 for fungi (SDB). Exactly 0.4 mL of the test compound solution was added to 1.6 mL of the seeded broth to form the first dilution. One milliliter of this was diluted with a further 1 mL of seeded broth to give the second dilution and so on till six such dilutions were obtained. A set of assay tubes containing only the seeded broth was kept as a control. The tubes were incubated in biochemical oxygen demand (BOD) (Sigma Instruments, Chennai, India) incubators at 37 ± 1°C for bacteria and 28 ± 1°C for fungi. MICs were recorded by visual observations after 24 h (for bacteria) and 72–96 h (for fungi) of incubation. Penicillin was used as the standard for bacterial studies and amphotericin B was used as the standard for fungal studies.

Results and discussion

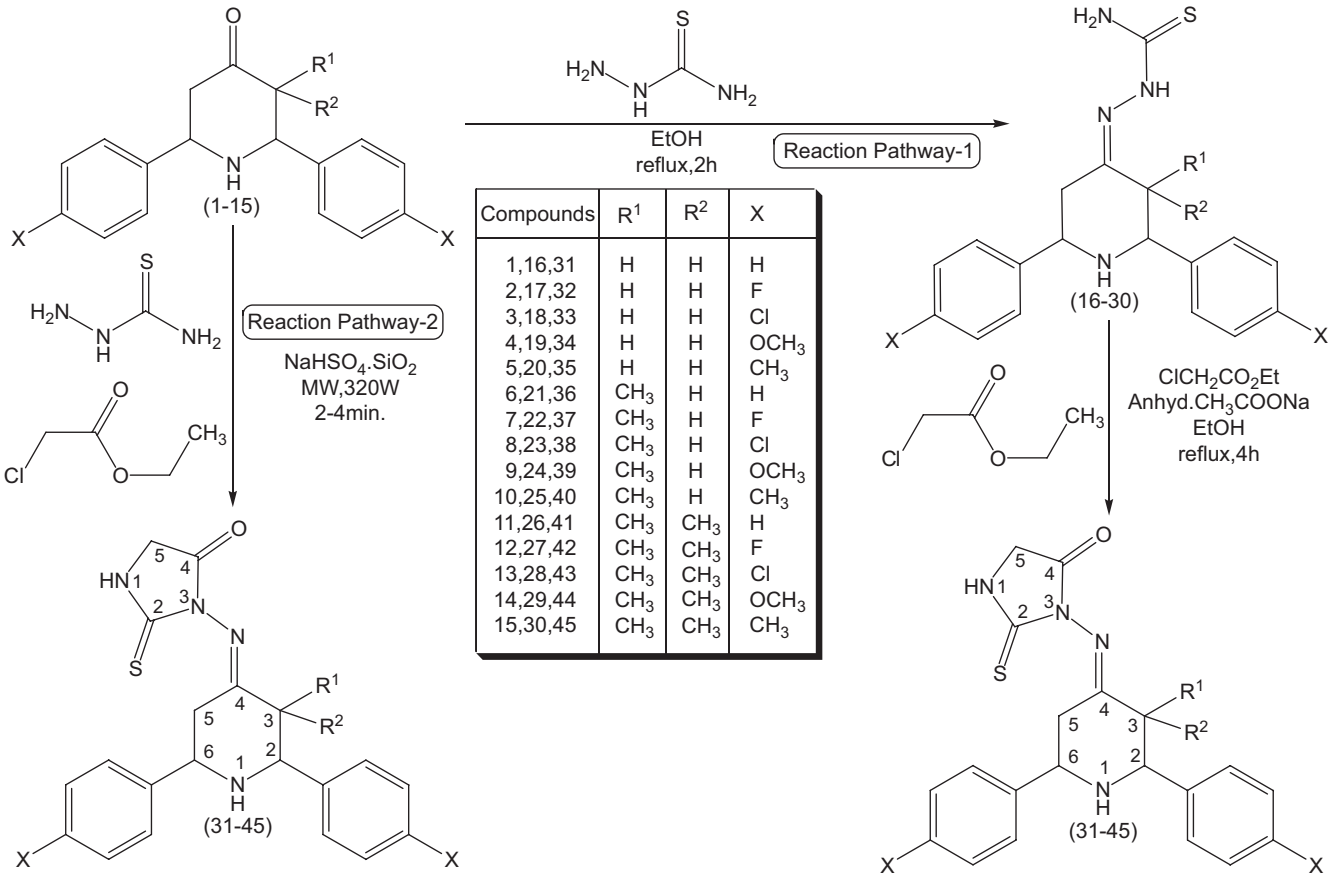
In the current work, the classical synthetic strategy adopted to obtain the a new series of hybrid heterocycles comprising both piperidine and thiohydantoin nuclei namely 3-(3-alkyl-2,6-diarylpiperin-4-ylidene)-2-thioxoimidazolidin-4-ones **31**–**45** was synthesised by the treatment of the respective thiosemicarbazones **16**–**30** with chloroethyl acetate and anhydrous sodium acetate in refluxing ethanol for 4 h. The synthetic route for the formation of compounds **31**–**45** is given in Scheme 1. The physical and analytical data is in Table 1. In order to avoid the use of solvent, to minimise the reaction time and to improve the yield of the product, a 'one-pot' reaction procedure was developed by the treatment of 3-alkyl-2,6-diarylpiperidin-4-ones **1**–**15**, thiosemicarbazide and chloroethyl acetate in the ratio of 1:1:1 the same reaction was also performed under microwave irradiation in a scientific microwave oven using a catalytic amount of $NaHSO_4.SiO_2$ (25 mg) to afford the title compounds in high yields in dry media. The $NaHSO_4.SiO_2$ catalyst was shown to be one of the most efficient MW absorbers with a very high specificity to MW heating. It was able to reach a temperature of 110°C after 3 minutes of irradiation in a domestic oven (320 W). A mere 25 mg of $NaHSO_4.SiO_2$ catalyst to 0.01 moles of substrates was the most acceptable ratio in terms of efficiency and safety; a power level of 320 watts was the most suitable. Moreover, the catalyst assists in the removal of water molecules and facilitates the condensation reaction to form the title compounds.

The conversion of 3-alkyl-2,6-diarylpiperidin-4-ones **1**–**15** into 3-(3-alkyl-2,6-diarylpiperin-4-ylidene)-2-thioxoimidazolidin-4-ones **31**–**45** using this method was followed via the 3-alkyl-2,6-diarylpiperidin-4-one thiosemicarbazone derivatives **16**–**31**. In the first step, 3-alkyl-2,6-diarylpiperidin-4-ones were converted to their respective thiosemicarbazones and rapidly rearrange to give 3-(3-alkyl-2,6-diarylpiperin-4-ylidene)-2 -thioxoimidazolidin-4-ones **31**–**45** in the second step. The attempt to isolate the respective thiosemicarbazones from the reaction mixture was unsuccessful. The formation of 3-(3-alkyl-2,6-diarylpiperin-4-ylidene)-2-thioxoimidazolidin-4-ones **31**–**45** via the thiosemicarbazones were confirmed by the same kind of reactions carried out using the $NaHSO_4.SiO_2$ catalyst and 3-alkyl-2,6-diarylpiperidin-4-ones thiosemicarbazones **16**–**30** and under microwave irradiation for 2–4 min. The products formed from the above two methods were found to be the same. The structures of all the synthesised compounds 3-(3-alkyl-2,6-diarylpiperin-4-ylidene)-2 -thioxoimidazolidin-4-ones **31**-**45** were characterised on the basis of their mps, elemental analysis, FT-IR, MS, one-dimensional NMR (^1H, ^{13}C), two dimensional HOMOCOSY and NOESY spectra which were in good agreement with the proposed structures.

Spectral assignments for the newly synthesised compound 31

In order to investigate the spectral assignments, compound **31** was chosen as a representative compound. The IR spectrum of 3-(2,6-diphenylpiperidin-4-ylideneamino)-2-thioxoimidazolidin-4-one **31** shows characteristic frequencies at 1728, 1215 cm^{-1} were due to the presence of carbonyl and thiocarbonyl groups. The absorption frequencies shown in the region of 3300-3426 cm-1 suggested the presence of -NH groups. In addition, the absorption frequency at 1635 cm^{-1} was due to C=N stretching vibration. The mass spectrum of compound **31** shows a molecular ion peak at an *m/z* of 365.25 (M+•+1) which was consistent with the proposed molecular formula of **31**. The elemental analysis (Ccal 65.87, Cobs 65.91; Hcal 5.53, Hobs 5.5; Ncal 15.37, Nobs 15.33) were consistent with the proposed molecular formula ($C_{20}H_{20}N_{40}S$) of **31**.

The assignment of signals in the ^1H NMR spectrum of compound **31** were based on total widths and spin multiplicities. There were two double doublet centered at 3.9 ppm and 4.17 ppm. Each signal corresponded to



Compounds	R^1	R^2	X
1,16,31	H	H	H
2,17,32	H	H	F
3,18,33	H	H	Cl
4,19,34	H	H	OCH_3
5,20,35	H	H	CH_3
6,21,36	CH_3	H	H
7,22,37	CH_3	H	F
8,23,38	CH_3	H	Cl
9,24,39	CH_3	H	OCH_3
10,25,40	CH_3	H	CH_3
11,26,41	CH_3	CH_3	H
12,27,42	CH_3	CH_3	F
13,28,43	CH_3	CH_3	Cl
14,29,44	CH_3	CH_3	OCH_3
15,30,45	CH_3	CH_3	CH_3

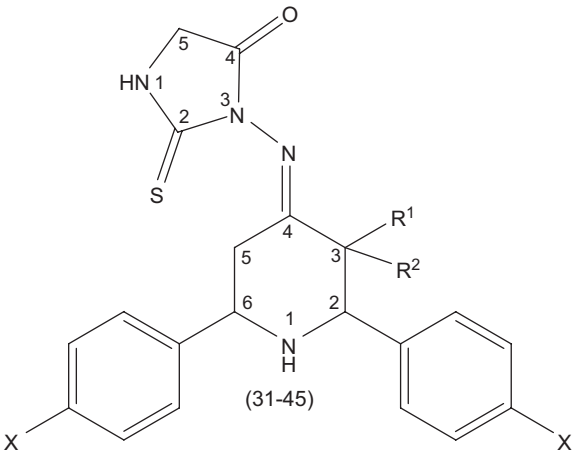
Scheme 1. A facile synthetic route for the synthesis of 3-(3-substituted-2,6-diarylpiperidin-4-ylideneamino)-2-thioxoimidazolidin-4-ones.

one proton. These two signals were due to the benzylic protons H_{2a} and H_{6a} (3.9/H_{2a}, 4.17/H_{6a}). Two coupling constants were extracted from the double doublet at 3.88–3.92 and the values were found to be 11.76 Hz and 3.08 Hz. The lower value was due to the vicinal coupling of $J_{2a,3e}$ and the higher value corresponds to the *trans* coupling of $J_{2a,3a}$. The two coupling constants were calculated from the double doublet at 4.15–4.19 ppm and the values were 11.88 Hz and 3.20 Hz. The lower value was due to the vicinal coupling of $J_{6a,5e}$ and the higher value is due to the *trans* coupling of $J_{6a,5a}$. The double doublets observed at 2.41–2.37 ppm and 3.62–3.66 ppm were due to the equatorial methylenic protons H_{3e} and H_{5e} respectively. A double doublet was expected for the axial methylenic proton H_{5a}, but a multiplet was obtained at 2.43–2.52 ppm corresponding to the H_{5a} proton. A double doublet at 2.37–2.41 ppm had two coupling constants and the coupling values were 2.96 Hz and 13.64 Hz. The lower value corresponds to vicinal coupling of $J_{3e,2a}$ and the higher value corresponds to geminal coupling of $J_{3e,3a}$. The two coupling constant values were extracted from the double doublet at 3.62–3.66 ppm. The lower value 2.96 Hz was due to vicinal coupling of $J_{5e,\ 6a}$ and the higher value 13.52 Hz is due to geminal coupling of $J_{5e,\ 5a}$. A sharp singlet observed at 3.80 ppm was due to the methylene protons of the imidazolidine moiety. A double doublet was expected for the axial methylenic proton H_{3a}. but a multiplet was obtained at 1.97–2.05 ppm corresponding to the H_{3a} proton. The NH proton of the piperidone moiety was observed as a broad singlet at 2.83 ppm. The broad signal at 11.78 ppm was due to the NH proton of the imidazolidine moiety. A multiplet appeared in the range of 7.23 –7.5 ppm due to the aromatic ring protons at the C-2 and C-6 positions.

In the ^{13}C NMR spectrum of **31**, resonances in the aliphatic range 29.62, 37.3, 60.23, 61.12, 43.64 ppm were observed. The signals appeaing at 29.62 and 37.3 ppm were due to the C-3 and C-5 carbons respectively. Among the signals at 60.23 ppm and 61.12 ppm for the benzylic carbons, the one at 60.23 ppm was due to C-2 and the signal at 61.12 ppm was due to C-6 carbon. The ^{13}C resonance at 43.64 ppm must be due to the methylene carbon of the imidazolidine moiety (at C-5). The signal at 163.19 ppm was conveniently assigned to the C=N carbon of the piperidone moiety. The ^{13}C resonance of the carbonyl carbon and the thiocarbonyl carbon at the C-4 and C-2 of the midazolidine moiety appeared at 167.63 and 173.87 ppm (C=S/173.87; C=O/167.63) respectively. The *ipso* carbons appeared at 144.01 and 144.16 ppm. The signals appearing in the region of 126.51–128.15 were due to the aromatic carbons in the two phenyl rings at the C-2 and C-6 positions. All the above mentioned assignments were further confirmed by HOMOCOSY and NOESY spectra.

In the HOMOCOSY spectrum of **31**, the signal at 3.9 ppm showed cross peaks with the signals at 2.01 ppm

Table 1. Physical and analytical data of compounds **31**–**45**.



Compound	R^1	R^2	X	Yield Δ (MW) (%)	mp (°C)	Elemental analysis (%) C Found (calculated)	H Found (calculated)	*N* Found (calculated)	*m/z* $(M+1)^+$. Molecular formula
31	H	H	H	74 (90)	108	65.85 (65.91)	5.45 (5.53)	15.3 (15.37)	365.2 $C_{20}H_{20}N_4OS$
32	H	H	F	78 (92)	103	59.91 (59.99)	4.44 (4.53)	13.9 (13.99)	400.4 $C_{20}H_{18}F_2N_4OS$
33	H	H	Cl	67 (82)	167	55.39 (55.43)	4.15 (4.19)	12.81 (12.93)	432.3 $C_{20}H_{18}Cl_2N_4OS$
34	H	H	OCH_3	72 (90)	158	62.2 (62.24)	5.66 (5.7)	13.17 (13.20)	424.0 $C_{22}H_{24}N_4O_3S$
35	H	H	CH_3	70 (88)	142	67.3 (67.32)	6.1 (6.16)	14.23 (14.27)	392.3 $C_{22}H_{24}N_4OS$
36	CH_3	H	H	75 (95)	171	66.6 (66.64)	5.82 (5.86)	14.76 (14.8)	378.2 $C_{21}H_{22}N_4OS$
37	CH_3	H	F	78 (92)	182	60.81 (60.85)	4.82 (4.86)	13.48 (13.52)	414.1 $C_{21}H_{20}F_2N_4OS$
38	CH_3	H	Cl	75 (85)	187	56.35 (56.38)	4.4 (4.51)	12.43 (12.52)	446.3 $C_{21}H_{20}Cl_2N_4OS$
39	CH_3	H	OCH_3	72 (90)	182	62.95 (62.99)	5.95 (5.98)	12.68 (12.78)	438.5 $C_{23}H_{26}N_4O_3S$
40	CH_3	H	CH_3	70 (94)	136	67.9 (67.95)	6.41 (6.45)	13.75 (13.78)	406.4 $C_{23}H_{26}N_4OS$
41	CH_3	CH_3	H	76 (96)	148	67.28 (67.32)	6.1 (6.16)	14.21 (14.27)	392 $C_{22}H_{24}N_4OS$
42	CH_3	CH_3	F	77 (95)	134	61.64 (61.67)	5.11 (5.17)	13.03 (13.08)	428.2 $C_{22}H_{22}F_2N_4OS$
43	CH_3	CH_3	Cl	75 (90)	176	57.21 (57.27)	4.78 (4.81)	12.11 (12.14)	460 $C_{22}H_{22}Cl_2N_4OS$
44	CH_3	CH_3	OCH_3	72 (85)	132	63.6 (63.69)	6.1 (6.24)	12.31 (12.38)	452.4 $C_{24}H_{28}N_4O_3S$
45	CH_3	CH_3	CH_3	71 (88)	124	68.44 (68.54)	6.65 (6.71)	13.2 (13.32)	420.1 $C_{24}H_{28}N_4OS$

and 2.39 ppm. The signal at 4.17 ppm showed cross peaks with the signals at 2.48 and 3.64 ppm. Consequently, the signal at 3.9 ppm must be due to the benzylic proton H_{2a}. Since this can have coupling only with the H_{3e} and H_{3a} protons, the two signals at 2.01 ppm and 2.39 ppm were assigned to the H_{3a} and H_{3e} protons respectively. The signal at 4.17 ppm must be due to the benzylic proton H_{6a}, since this can have coupling with the H_{5a} and H_{3e} protons. The two signals at 2.48 and 3.64 ppm were assigned to the H_{5a} and H_{5e} protons respectively. The individual assignments can be made by using its NOESY spectrum.

In the NOESY spectrum of **31**, the signals of the benzylic proton (H_{2a},H_{6a}) have a strong nOe with the signals of the methylene protons (H_{3e} and H_{5a} proton). Hence the signal at 3.9 ppm has a strong nuclear Overhauser effect (nOe) with the signal at 2.39 ppm. The signal at 4.17 ppm has a strong nOe with the signal 2.48 ppm. From this it was concluded that the signal at 3.9 ppm must be due to the benzylic proton H_{2a}. The signal at 4.17 ppm must be due to the benzylic proton H_{6a}. The signals at 2.39 ppm and 2.48 ppm must be due to the H_{3e} and the H_{5a} proton respectively. Moreover, an interesting observation from the NOESY spectrum of compound **31** was that the signal at 11.78 ppm had a strong nOe with the signal at 2.01 ppm. Hence, the signal at 11.78 must be due to the NH proton of the imidazolidine moiety and the signal at 2.01 ppm must be due to the H_{3a} proton. The signal at 3.80 ppm has strong nOe with the signals at 2.01 ppm and 2.39 ppm. The signal at 3.8 ppm must be due to the methylene proton of the imidazolidine moiety. The signal at 2.39 ppm was due to the H_{3e} proton. From the NOESY spectrum, we concluded that the imidazolidine moiety of **46** presents toward the H_{3a} and H_{3e} protons of the piperidone moiety of **31**. From the view of nOes of -NH and CH_2 protons of the imidazolidine moiety, the -NH signal (11.78 ppm) and CH_2 signal (3.8 ppm) must be very close to the methylene protons of the piperidone moiety at the C-3 position.

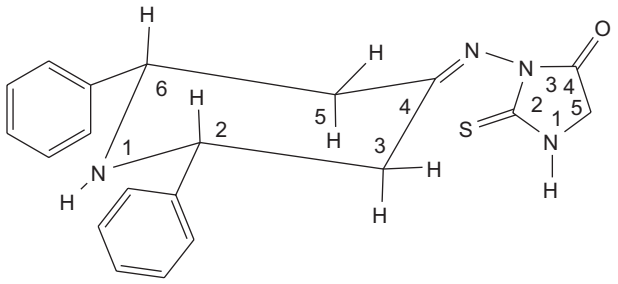


Figure 1. Chair conformation for compound **31**.

Therefore, taking into account the HOMOCOSY and NOESY correlations for compound **31**, the tentative assignments made for its ring and substituent protons were confirmed. It has been shown that the 2,6-diarylpiperidin-4-one ring mostly and favourably adopts the chair conformation with all its substituents at an equatorial disposition [12]. Moreover the equatorial disposition of the phenyl group at the C-2 and C-6 makes the chair conformation more rigid thereby preventing interconvertion of one chair form into another. Hence, based on the obtained chemical shifts and coupling constant values, the normal chair conformation (Figure 1) was proposed for compound **31**.

Two dimensional HOMOCOSY and NOESY NMR spectral assignments for the newly synthesised 3-(3-methyl-2,6-diphenylpiperidin-4-ylideneamino)-2-thioxoimidazolidin-4-one 36

In the HOMOCOSY spectrum of **36**, the double doublet at 3.56 ppm had cross peaks with the signals at 2.08 ppm and 2.74 ppm and vice versa. This mutual correlation clearly showed that these two signals must be due to the H_{5a} and H_{5e} proton. The signal at 3.56 ppm must be due to the benzylic proton H_{6a}. Besides, the doublet at 3.46 ppm had a cross peak with the signal at 2.59 ppm and 0.78 ppm. Therefore, the signal at 3.46 ppm must be due to the benzylic proton H_{2a} and the signal at 2.59 ppm should be due to the H_{3a} proton. Likewise, the methyl proton doublet at 0.78 ppm had a strong cross peak with the signal at 2.59 ppm. Hence, the signal at 2.59 ppm must be due to the H_{3a} proton. The individual assignments can be made by using the NOESY spectrum.

From the NOESY spectrum of compound **36**, it was interesting to note that the signal at 0.78 ppm of C-3 methyl protons had strong nOe with the benzylic proton signal at 3.46 ppm and the methine proton signal at 2.59 ppm. It further confirmed the assignment of the doublet at 3.46 ppm to the benzylic proton H_{2a} and the signal centred at 2.59 ppm to the methine proton using the HOMOCOSY spectrum of the same compound. The benzylic proton signal at 3.46 ppm had a nOe with the methine proton signal at 2.59 ppm and the C-3 methyl proton signal at 0.78 ppm. Hence, the signals at 2.59 ppm and 3.46 ppm must be due to the H_{3a} and H_{2a} protons respectively. The double doublet at 3.56 ppm had a cross peak with the methine proton signals at 2.08 ppm and 2.74 ppm. This suggested that the signal at 2.08 ppm was due to the H_{5a} proton and the signal at 2.74 ppm must be due to the H_{5e} proton. This showed that the signal at 3.56 ppm should be due to the benzylic proton H_{6a}. The signal at 3.76ppm had a strong nOe with the signal at 2.08 ppm. Hence the signal at 3.76 ppm must be due to the methylene proton of the imidazolidine moiety. The NH proton signal at 11.71 ppm of the imidazolidine moiety had a strong nOe with the methine proton signal at 2.74 ppm. Hence, the NH and CH_2 protons of the imidazolidine moiety must be very close to the methylene protons at C-5 position of piperidone moiety. Therefore, with reference to HOMOCOSY and NOESY correlations in compound **36**, the assignments made for its ring and substituent protons were confirmed. Hence based on the obtained chemical shifts and coupling constant values, a normal chair conformation (Figure 2) was proposed for compound **36**.

Two dimensional HOMOCOSY and NOESY NMR spectral assignments for the newly synthesised 3-(3,3-dimethyl-2,6-diphenylpiperidin-4-ylideneamino)-2-thioxoimidazolidin-4-one 41

Two singlets of the two methyl proton at 0.97 and 1.19 ppm had a cross peak with the signal at 3.72 ppm. Hence, the signal at 3.72 ppm must be due to the H_{2a} proton. The double doublet at 4.15ppm had cross peaks with the signal at 2.54ppm and vice versa. This mutual correlation clearly showed that the signal at 4.15ppm was due to the H_{6a} proton and the signal at 2.54 ppm must be due to the H_{5e} proton. The individual assignments can be made by using its NOESY spectrum.

From the NOESY spectrum of compound **41,** it was interesting to note that the two methyl protons at 0.97 ppm and 1.19 ppm had a nOe with protons at 3.72 ppm. This shows that the signal at 3.72 ppm should be due to the H_{2a} proton. The double doublet at 4.15 ppm had a strong nOe with the proton at 2.54 ppm. This revealed that the signal at 2.54 ppm must be due to the H_{5e} proton. In addition, the signal at 3.91 ppm had a strong nOe with the proton signal at 2.54 ppm and a weak nOe with the proton signal at 3.21 ppm. Hence, the signal at 3.91 ppm must be due to the methylene proton of the imidazolidine moiety. The signal at 3.21 ppm must be due to the H_{5a} proton. The NH proton signal (11.8 ppm) of the imidazolidine moiety had a strong nOe with the methine proton signals at 2.54 and 3.21 ppm. This showed clearly that the NH and CH_2 protons of the imidazolidine moiety must be very close to the methylene protons at the C-5 position of piperidone moiety. Therefore, with reference to HOMOCOSY and NOESY correlations in compound **41,** the assignments made for its ring and substitutent protons were confirmed. Hence based on the obtained chemical shifts and coupling constant values, a normal chair conformation (Figure 3) was proposed for compound **41**.

Antibacterial activity

New 3-(3-alkyl-2,6-diarylpiperin-4-ylidene)-2-thioxoimidazolidin-4-ones **31**–**45** were tested for their antibacterial activity *in vitro* against clinically isolated bacterial strains namely

B. subtilis, M. luteus, S. typhii, S. paratyphii B, S. felxneri and *P. vulgaris*. Penicillin was used as the standard drug. The minimum inhibitory concentration (MIC) in μg/mL values was reproduced in Table 2. Compound **31** which doesn't have a substituent at the *para* position of the phenyl ring at positions 2 and 6 of the piperidone ring exhibited antibacterial activity against *B. subtilis* at a MIC value of 100 μg/mL. Introduction of a methyl substituent at position 3 of the piperidone ring in compound **36** enhanced its activity with a MIC value of 12.5 μg/mL. Further introduction of a dimethyl substituent at position 3 of the piperidone ring in compound **41** enhanced the antibacterial activity against *B. subtilis* with a MIC value of 6.25 μg/mL. Compounds **32** and **33** which have electron withdrawing fluoro and chloro functional group at the *para* position of the phenyl rings at positions 2 and 6 of the piperidone ring respectively were inactive against *B. subtilis* and exhibited a MIC of 200 μg/mL. Similar results were observed for compounds **37**, **38**, **42** and **43** against *B. subtilis*. But introduction of electron donating methoxy and methyl functional group at the *para* position of the phenyl rings at positions 2 and 4 of the piperidone ring in compounds **34** and **35** showed moderate activity against *B. subtilis*. In addition to an electron donating methoxy and methyl functional group at the *para* position of the phenyl rings at positions 2 and 4 of the piperidone ring, a methyl substituent at position 3 of piperidone ring in compounds **39** and **40** were inactive against *B. subtilis*. Against *B. subtilis*, analogous results were observed in the dimethyl substituted compounds, **44** and **45**. Compound **31**, the methyl substituted compound **36** and the dimethyl substituted compound **41** none of which had a substituent at the *para* position of the phenyl rings at positions 2 and 6 of the piperidone ring exhibited antibacterial activity against *M. luteus* at a MIC value of 200 μg/mL. The introduction of an electron withdrawing fluoro and chloro functional group at the *para* position of the phenyl rings at positions 2 and 6 of the piperidone ring respectively in compounds **32** and **33** were active against *M. luteus* and exhibited a MIC of 12.5 μg/mL. Methyl substitution at position 3 of the piperidone ring for compounds **37** and **38** which also have electron withdrawing fluoro and chloro functional group at the *para* position of the phenyl rings at positions 2 and 6 of the piperidone ring respectively, were potent against *M. luteus* and exhibited a MIC of 6.25 μg/

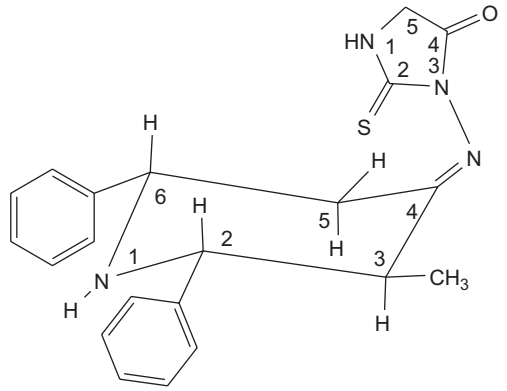


Figure 2. Chair conformation for compound **36**.

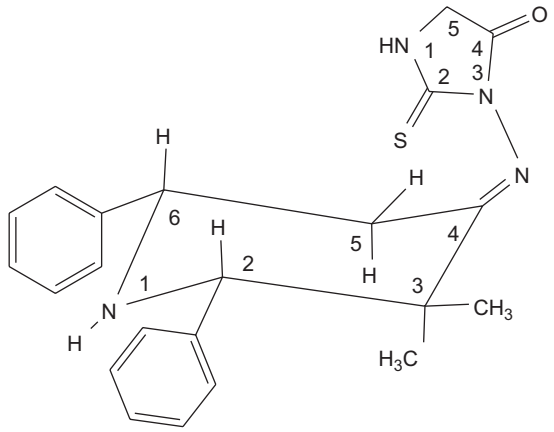


Figure 3. Chair conformation for compound **41**.

Table 2. *In vitro* antibacterial activity of compounds **31**–**45** against clinically isolated bacterial strains.

	Minimum inhibitory concentration (MIC) in μg/mL					
Compound	*B. subtilis*	*M. luteus*	*S. typhii*	*S. paratyphii B*	*S. felxneri*	*P. vulgaris*
31	100	200	'-'	200	50	100
32	200	12.5	12.5	25	200	50
33	200	12.5	25	25	100	50
34	25	50	100	100	12.5	25
35	25	100	100	50	12.5	25
36	12.5	200	'-'	200	50	200
37	100	6.25	12.5	12.5	100	25
38	100	6.25	12.5	12.5	100	25
39	200	25	50	200	12.5	12.5
40	200	50	50	100	25	12.5
41	6.25	200	'-'	'-'	100	50
42	200	25	6.25	6.25	200	12.5
43	'-'	25	6.25	6.25	200	12.5
44	100	50	25	100	6.25	6.25
45	100	50	25	100	12.5	6.25
Penicillin	25	12.5	25	25	25	12.5

'-' No inhibition even at a higher concentration of 200 μg/mL

Table 3. *In vitro* antifungal activity of compounds **31**–**45** against clinically isolated fungal strains.

	Minimum inhibitory oncentration (MIC) in μg/mL			
Compound	*A. niger*	*Mucor*	*Rhizopus*	*M. gypsuem*
31	200	100	200	200
32	25	6.25	100	100
33	25	12.5	200	100
34	50	100	6.25	12.5
35	50	100	6.25	6.25
36	'-'	100	50	50
37	12.5	6.25	200	'-'
38	12.5	6.25	200	200
39	50	200	12.5	25
40	50	200	25	25
41	100	100	100	100
42	6.25	12.5	100	50
43	12.5	12.5	100	50
44	100	50	200	100
45	100	50	200	100
Amphotericin B	25	25	50	25

'-', No inhibition even at a higher concentration of 200 μg/mL

mL whereas dimethyl substitution at position 3 of the piperidone ring for compounds **42** and **43** were inactive against *M. luteus*. Compound **34, 35**, methyl substituted compound **39, 40** and dimethyl substituted compound **44, 45** which all have an electron donating methoxy and methyl functional group at the *para* position of the phenyl rings at positions 2 and 6 of the piperidone ring exhibited moderate antibacterial activity against *M. luteus*. Against *S. typhii,* compounds **31**, **36** and **41** were inactive. The electron withdrawing substituted compounds of **32, 33, 37, 38, 42** and **43** showed potent activity against *S. typhii* at a MIC value of 12.5–6.25 μg/mL. The electron donating substituted compounds of **34, 35, 39, 40, 44** and **45** showed moderate activity against *S. typhii* at a MIC value of 100–25 μg/mL. The electron withdrawing substituted compounds of **37, 38, 42** and **43** were active against *S. paratyphii B*. The electron donating substituted compounds of **34, 35**, **39, 40, 44** and **45** were active against *S. felxneri*. Against *P.vulgaris* all the compounds exerted activity at a MIC value of 100–6.25 μg/mL.

Antifungal activity

The *in vitro* antifungal activity of the 3-(3-alkyl-2,6-diarylpiperin-4-ylidene)-2-thioxoimidazolidin-4-ones **31-45** was studied against clinically isolated the following fungal strains: *A. niger, Mucor, Rhizopus* and *M. gypsuem.* Fluconazole was used as a standard drug. Minimum inhibitory concentration (MIC) in μg/mL values are reproduced in Table 3. Compound **36** which doesn't have a substitutent at the *para* position of the phenyl rings at positions 2 and 4 of the piperidone ring but does have an electron donating methyl substituent at position 3 of the piperidone ring did not exert any antifungal activity against *A. niger* and *M. gypsuem* even at a high concentration of 200 μg/mL. The methyl substituted compounds of **37** and **38** with electron withdrawing fluoro and chloro functional groups at the *para* position of the phenyl rings at positions 2 and 6 of the piperidone ring exerted good activity at a MIC of 12.5 μg/mL against *A. niger*. The dimethyl substituted compounds of **42** and **43** with electron withdrawing fluoro and chloro functional groups at the *para* position of the phenyl rings at positions 2 and 6 of the piperidone ring exerted good activity at a MIC of 6.25 and 12.5 μg/mL respectively against *A. niger*. Against *Mucor*, the electron withdrawing fluoro and chloro substituted compounds **32, 33, 37, 38, 42** and **43** exhibited good antifungal activity at a concentration of 12.5-6.25 μg/mL. Compounds **34, 35, 39, 40, 44** and **45** which all have electron donating methoxy and methyl functional group at the *para* position of the phenyl rings at positions 2 and 6 of the piperidone ring exerted good activity at a MIC of 6.25 and 12.5 μg/mL against *Rhizopus*. Similarly compounds **34** and **35,** which have electron donating methoxy and methyl functional group at the *para* position of the phenyl rings at positions 2 and 6 of the piperidone ring exerted excellent antifungal activity against *M. gypsuem* at a MIC of 6.25 and 12.5 μg/mL.

Conclusion

The 3-(3-Alkyl-2,6-diarylpiperin-4-ylidene)-2-thioxoimidazolidin-4-ones, a class of novel hybrid heterocyclic compounds can be synthesised from various 3-alkyl-2-,6-diphenylpiperidin-4-one thiosemicarbazones, anhydrous sodium acetate and chloroethyl acetate in refluxing ethanol and a comparative study was also carried out under microwave irradiation. The synthesised compounds were characterised by mps, elemental analysis, MS, FT-IR, one-dimensional NMR (^{1}H, D_2O exchanged ^{1}H & ^{13}C), two dimensional HOMOCOSY and NOESY spectroscopic data. The *in vitro* microbiological screening studies carried out to evaluate the antibacterial and antifungal potencies of the newly synthesised 3-(3-alkyl-2,6-diarylpiperin-4-ylidene)-2-thioxoimidazolidin-4-ones **31**–**45** are shown in Table 2 and Table 3. A close inspection of the *in vitro* antibacterial and antifungal activity profile in differently electron withdrawing (F and Cl) and electron donating (OCH_3 and CH_3) functional groups substituted phenyl rings of novel 3-(3-alkyl-2,6-diarylpiperin-4-ylidene)-2-thioxoimidazolidin-4-ones **31**–**45** exerted strong antibacterial activity against all the clinically isolated tested bacterial and fungal strains namely: *B. subtilis, M. luteus, S. typhii, S. paratyphii B, S. felxneri, P. vulgaris, A. niger, Mucor, Rhizopus* and *M. gypsuem.* These observations may promote further development of our research into this group of pyrido 2-thioxoimidazolidin-4-ones and may lead to the development of compounds with a better pharmacological profile than the standard antibacterial and antifungal drugs.

Acknowledgements

The authors would like to thank the NMR Research Centre, Indian Institute of Science, Bangalore for recording spectra.

Declaration of interest

V. Kanagarajan is grateful to the Council of Scientific and Industrial Research (CSIR), New Delhi, Republic of India for providing financial support in the form of CSIR-Senior Research Fellowship (SRF) in Organic Chemistry. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Cherouvrier JR, Carreaux F, Bazureau JP. Reactivity of 2-thiohydantoins towards various electrophilic reagents: Applications to the synthesis of new 2-ylidene-3,5-dihydro-4H-imidazol-4-ones. Molecules 2004;9:867–875.
2. Wang ZD, Sheikh SO, Zhang Y. A simple synthesis of 2-thiohydantoins. Molecules 2006;11:739–750.
3. Gopalakrishnan M, Kanagarajan V, Thanusu J. Novel one-pot synthesis of 1,2,4-triazolidin-3-thiones comprising piperidine moiety. Green Chem Lett Rev 2008; 1:241–246.
4. Gopalakrishnan M, Sureshkumar P, Kanagarajan V, Thanusu J, Govindaraju R. A simplified green chemistry approaches to organic synthesis in solid media. activated fly ash, an industrial waste (pollutant) as an efficient and novel catalyst for some selected organic reactions in solvent-free conditions under microwave irradiation. Arkivoc 2006;13:130–141.
5. Gopalakrishnan M, Sureshkumar P, Kanagarajan V, Thanusu J, Govindaraju R, Ezhilarasi MR. Microwave-promoted facile and rapid solvent-free procedure for the efficient synthesis of 3,4-dihydropyrimidin-2(1H)-ones and – thiones using ZrO_2/SO_4^{2-} as a reusable heterogeneous catalyst. Lett Org Chem 2006;3:484–488.
6. Gopalakrishnan M, Sureshkumar P, Kanagarajan V, Thanusu J. Aluminium metal powder (atomized) catalyzed Friedel-Crafts acylation in solvent-free conditions: A facile and rapid synthesis of aryl ketones under microwave irradiation. Catalysis Commun 2005;6:753–756.
7. Gopalakrishnan M, Sureshkumar P, Kanagarajan V, Thanusu J, Govindaraju R. Silica gel supported sodium hydrogen sulfate as an efficient and reusable heterogeneous catalyst for the synthesis of imines in solvent-free conditions under microwave irradiation. J Chem Res 2005;5:299–303.
8. Gopalakrishnan M, Sureshkumar P, Kanagarajan V, Thanusu J. Organic synthesis in solid media. Alumina supported sodium hydrogen sulfate as an effective and reusable catalyst for 'one-pot' synthesis of amides from ketones in dry media under microwave irradiation. Lett Org Chem 2005;2:444–446.
9. Gopalakrishnan M, Sureshkumar P, Kanagarajan V, Thanusu J. Design, 'one-pot' synthesis, characterization, antibacterial and antifungal activities of novel 6-aryl-1,2,4,5-tetrazinan-3-thiones in dry media. J Sulfur Chem 2007;8:383–392.
10. Gopalakrishnan M, Sureshkumar P, Thanusu J, Kanagarajan V, Govindaraju R, Jayasri G. A convenient 'one-pot' synthesis and *in vitro* microbiological evaluation of novel 2,7-diaryl-[1,4]-diazepan-5-ones. J Enz Inhib Med Chem 2007;22:709–715.
11. Gopalakrishnan M, Thanusu J, Kanagarajan V. Heterogeneous $NaHSO_4.SiO_2$ catalyzed 'one-pot' synthesis and in vitro antibacterial and antifungal activities of pyridino-1,2,3-thiadiazoles. J Sulfur Chem 2008;29:179–185.
12. Baliah V, Jeyaraman R, Chandrasekaran L. Synthesis of 2,6-disubstituted piperidones, oxanes and thianes. Chem Rev 1983;83:379–423.
13. Noller CR, Baliah V. The preparation of some piperidine derivatives by the mannich reaction. J Am Chem Soc 1948;70:3853–3854.
14. Angle SR, Breitenbucher JG. In: Atta-ur-Rahman ed. Studies in Natural Products Chemistry, Stereoselective Synthesis Vol. 16 New York: Elsevier, 1995:453.
15. Dhar MH, Dhar MM, Dhawan BN, Mehrotra BN, Ray C. Screening of Indian plants biological activity. Part I. Indian J Exp Biol 1968;6: 232–247.