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Synthesis, Antifungal Activity and Molecular Docking Studies on *N*-(Substituted-benzylidineamine)-3- cycloalkylidine-thiosemicarbazide Derivatives

Sudha Vengurlekar[1]*, Rajesh Sharma[1], PiyushTrivedi[2]

***Corresponding author:**

Sudha Vengurlekar

[1]School of Pharmacy, Takshila Campus, Devi Ahilya Vishwavidyalaya (DAVV), Indore (MP), India
[2]Rajiv Gandhi Proudyogiki Vishwavidyalaya (RGPV), Bhopal (MP), India

Abstract

A series of substituted *N*-(Benzylidineamine)-3-cycloalkylidine-thiosemicarbazide derivatives have been synthesized, characterized and evaluated for their antifungal and antibacterial activities. The newly synthesized compounds were characterized by IR, NMR, Mass and elemental analysis. All compounds were tested for antifungal and antibacterial activities. The preliminary results revealed that some of the compounds exhibited promising antifungal activities. Among the tested compounds, compound 14 (MIC 8 µg/mL) and 25 (MIC 8 µg/mL) were the most effective against *C. Tropicalis.* Important SAR information was also gathered. Molecular modeling investigations showed that the active compounds may interact at the active site of the fungal cytochrome P450-dependent sterol 14 - demethylase (CYP51) in the sterol biosynthesis pathway.

Keywords: *N*-(Benzylidineamine)-3-cycloalkylidine-thiosemicarbazide; Antifungal; Antibacterial; Molecular modeling; CYP51; Minimum inhibitory concentration.

Introduction

Thiosemicarbazide derivatives are important class of molecules with a large spectrum of biological properties. These compounds have been studied as anti-tubercular [1], anti-bacterial [2], anti-malarial [3], anti-leprosy [4], anti-parasitic [5] andantineoplastics [6].Antituberculer activity of cycloalkane analogues of thiosemicarbazidesis well documented [7].Cyclic analogues (cycloalkyl: cyclopentyl, cyclohexyl*etc.*) were also found to be active against several species of candida (*C. albicans* and *C. krusei*) [8]. The effective role of azomethine linkage [9] and –N=C=S linkage [10] has been well stressed and reported to be responsible for various biological activities in many compounds containing sulphur, such as dithiocarbamates, thiourea and thiosemicarbazides [11]. In a previous investigation on isothiosemicarbazone derivatives an interesting activity against both fungi and bacteria has been shown [12–15]. Prompted by the observed biological activities of the above mentioned derivatives we have designed and synthesized some structurally new thiosemicarbazide derivatives, bearing cycloalkane, thiol and azomethine functionality in the same molecule, as antifungal and antibacterial agents. Influence of structural changes has been investigated and on the basis of the obtained data some structure–activity relationships have been pointed out.

Materials and methods

Antifungal susceptibility testing

Evaluations of the susceptibility of fungal strains were performed using macro tube dilution method as per NCCLS guidelines [16]. The fungi used as inocula were grown overnight on sabouraud dextrose agar (SDA) at 25±1 C. To 5ml of SDA, 0.2 ml of culture was added and inoculated followed by incubation till it reached the turbidity equal to that of the standard 0.5 McFarland solution in 0.9 % (w/v)NaCl at 600nm which was equivalent to 10^6– 10^8 CFU/ml. A stock solution of 10 mg/mL was prepared in dimethyl sulfoxide (DMSO, Sigma) for the various synthesized compounds and for fluconazole (FLC) and griseofulvin (GRS), which were used as control. Two fold dilutions of test compounds from 64 to 0.25 µg/mL were prepared with the suspension of the inoculum. The micro dilution tubes, which contained 0.1 mL of the serially diluted drug, were inoculated with 0.1 mL of the resulting suspension. The final inoculum concentration after dilution with the drug suspension was 10^3/10^4 cells per mL. Two tubes containing the drug-free medium and inoculum were used as controls. The inoculated tubes were incubated at 35±1 C for 48h *(Candida* sp.) and 72 h *(A. niger*) in ambient air. The growth in each tube was then visually estimated. The minimum inhibitory concentrations (MIC) were determined visually, and were defined as the lowest concentration of a compound that inhibits growth of the organism as detected visually.

Antibacterial activity

Evaluations of the susceptibility of bacterial strains were made by the macro tube dilution method as per NCCLS guidelines [16]. Mueller-Hinton broth was used as the test medium. An inoculum of approximately $5x10^5$ CFU cm^{-3} was used. Serial twofold dilutions of the test compounds (64- 0.25 µg/mL) and extra dilutions (0.12-0.015 µg/mL) for antibiotic standard was prepared. Tubes were incubated for 24hrs at 37±1 C in an ambient air incubator. The lowest concentration of the test compounds inhibiting visible growth was taken as the MIC value.

Molecular modeling

Insilico homology modeling of 14- sterol demethylase (CYP51) of A. fumigatus

The 3D structure of the CYP51 from fungus *Aspergillus fumigates* (AFCYP51) have been constructed through homology modeling. Homology modeling or comparative modeling is predicting the protein 3D structure with the help of a close or homologue or known structure from the same family of the target protein. Amino acid sequences retrieved from swissprot/uniprot, provided descriptions of a non redundant set of proteins including their function, domain structure, posttranslational modifications and variants. Sequence of CYP51 of *A. Fumigatus* (AFCYP51) protein sequence (Accession num: AAK736590) was obtained from National Centre for Biotechnology Information (NCBI) as query sequence with total length of 461 amino acid. The selection of active site residues in the reference protein (MT-CYP51), was done by identifying residues in the substrate binding cavity and within 8 A from the heme as depicted in literature [17].

Template for modeling

Standalone blastp search was performed for finding similar structure entry in PDB database from ftp download available on ftp://ftp.ncbi.nih.gov/blast/db/FASTA/pdbaa.gz. A pair wise alignment has been performed using Blastp [18] against the target sequence of CYP51 of A. Fumigatus(AFCYP51).X-ray crystal structure of CYP51 (Pdb Code: 3LD6) selected for backbone alignment with domains identified in secondary structure studies determined, available on http://www.rcsb.org/pdb/explore/explore.do?structureId= 3LD6.

Comparative modeling

For modeling AFCYP51, ICM Molsoft has been adopted. ICM Molsoft algorithm contains robust modeling tools and high levels of accuracy with fast model building [19]. After initial alignment of query polypeptide chain on template backbone, side chain torsion angles were optimized using simultaneous global optimization of the energy for all non-identical residues. ICM Biased Probability Monte Carlo (BPMC) optimization facilitates conformational modeling through defining internal coordinates [20]. Extensive force field terms are conjoined and side entropy parameters proved ICM towards accuracy acceptance in CASP2 protein modeling competition [21, 22].Sequence of AFCYP51 is aligned on backbone of 3LD6 structure and comparative modeling was performed in ICM Molsoft. Energy minimization for modeled structure thermodynamically proved by accepted structure with energy of -26693.057 KJ/Mol and RMS value $0.30A^0$.

Structure validation using PROCHECK and standard drug FLC

Structure validation of enzyme structure modeled above is processed using PROCHECK which determine stereochemical aspects along with main chain and side chain parameters with comprehensive analysis [23]. Optimization and analysis of bond length and bond angles is referred from Cambridge Structural Database, CSD after studying 100,000 structures [24]. Main-chain parameters and side-chain parameters calculated at 2.0 Ang. of resolution signifies modeling of AFCYP51. Distances from planarity is found below 0.02 when plotted against amino acids frequency in sequence. The 3D modeled structure is validated by docking an X-ray structure of crystal structure of CYP51 from *Leishmaniainfantum* in complex with FLC (pdb code 3L4D).

Molecular docking

MolDock (MVD), based on a new heuristic search algorithm that combines differential evolution with a cavity prediction algorithm, was used for docking studies [25]. The hydrogens and bond orders were assigned to both ligand and protein molecules using automatic preparation function in MVD (grid resolution 0.20A˚). For each complex, charges and protonation states were assigned using docking scoring function, and all acyclic single bonds were set as flexible, except for bonds that only rotated hydrogens. During the docking experiments, structural water molecules were excluded. The population size, maximum iterations, scaling factor, and crossover rate were set to 50, 2000, 0.50, and 0.90, respectively. The highest ranked solution was compared with the known experimental structure using the standard Cartesian root-mean-square deviation (RMSD) measure (between similar atoms in the pose and experimental structure). Similar poses were clustered depending upon an RMSD threshold of 1.00A˚. The saved pose for the ligand–enzyme complex of each molecule was subjected to detailed 3D analysis for interactions at the enzyme active site. Structural illustrations were created by Molegro Virtual Docker under the 'Windows XP' operating system installed on an Intel Core 2 duo processor PC with a 2.8 MHz processor and 3GB RAM. The selection of active site residues in the reference protein (MT-CYP51), was done by identifying residues in the substrate binding cavity and within 8 A from the heme as per literature [26]. Putative active site residues in AFCYP51 were identified as Y107, L128, M129, E130, K133, F134, I135, L190, L290, M292, A293, G294, H296, S297, S300, I301, W304, H361, S362, S363, I364, S496.The structural models of selected 14 compounds with MICs, 2-16 µg/mL, were docked into the active site of the generated homology model of AFCYP51.

Synthesis

All reagents were purchased from Sigma- Aldrich and were used without further purification. All melting points of synthesized compounds were determined by open capillary method and are uncorrected. IR spectra were recorded with Shimadzu FT-IR-8400S spectrophotometer on KBr. Elemental analysis (%C, %H, %N, %O and %S) of the synthesized compounds were done on CorloErba 1108 Heracus instrument at Central Drug Research Institute (CDRI), Lucknow, India. The ^1H and ^{13}C NMR spectra were recorded on an Advance - 400 MHz Bruker, using TMS as an internal reference and mass spectra were obtained on a JMS-T100LC, Accu TOF (DARTMS)spectrophotometer at CDRI.

Synthesis of N-(3-substituted phenyl)-3-cycloalkylidene-thiosemicarbazide (1-3)

In a flask equipped with a reflux condenser, a mixture of cycloalkanone (0.018mol) and thiosemicarbazide (0.016 mol) were reacted in 80 ml of isopropyl alcohol in presence of catalytic amount of acetic acid. The suspension is refluxed until complete dissolution (30-90 mins). After completion of reaction, the reaction mixture was allowed to cool down to room temperature. The product is obtained as crystalline solid which is recrystallized from ethanol/ water-ethanol [27, 28].

N-(3-chlorophenyl)-3-cyclohexylidene-thiosemicarbazide (1)

IR ν cm^{-1}: 3383(NH); 3261 (NH); 3138 (CH); 2871 (CH); 1661 (C=S); 1607(C=N); 1445 (N-N); 830(C-Cl); 789,854 (Substituted Ar). ^1H NMR (DMSO-d6, 300 MHz, ppm): 1.2-1.3(p, 2H, CH_2); 1.7-1.8 (p, 4H, CH_2), 1.9-2.0(t, 4H, CH_2); 2.8 (s, NH); 7.0 (s, NH); 7.2-7.4 (Ar-H).^{13}C NMR (DMSO-d6, 75.4 MHz, ppm, DEPT): 187.94 (C=S);152.40(C=N);135.78 (ar-C-N); 131.22(Ar- C-Cl); 130-112.22 (Ar-4C); 77.32, 76.69, 34.07, 24.54, 22.65 (CH_2). Mass m/z: 281.07 (M+). Anal.Calcd. For C_{13} H_{16} N_3 S Cl (281.80): Calcd.:C, 55.41; H, 5.72; N, 12.58; S, 11.38; Found:C, 55.43; H, 4.69; N, 15.07; S, 11.18.

N-(3-chlorophenyl)-3-cyclopentylidene-thiosemicarbazide (2)

IR ν cm^{-1}: 3280, 3178(NH); 2989-2868 (CH),1575(C=S);1500(C=N); 1444(N-N);745 (C-Cl); 705,883 (Substituted Ar). ^1H NMR (DMSO-d6, 300 MHz, ppm): 1.4-1.8 (q, 4H, CH_2); 1.9-2.0(t, 4H, CH2); 2.2 (s, NH); 7.0 (s, NH); 7.2-7.5 (Ar-H).^{13}C NMR (DMSO-d6, 75.4 MHz, ppm, DEPT): 188.26 (C=S);152.19(C=N);135.87 (ar-C-N); 131.28 (Ar- C-Cl); 130.06-109.94 (Ar-4C); 77.32, 76.69, 39.34, 32.01, 22.67(CH_2). Mass m/z: 368 (M+). Anal.Calcd. For C_{12} H_{14} N_3 S Cl (267.78): Calcd.:C 53.82, H- 5.27, N-15.69, S-11.97; Found: C 53.64, H-5.14, N-15.61, S- 11.60.

N-(3-Chlorophenyl)-3-cycloheptylidenethiosemicarbazide(3)

IR ν cm^{-1}: 3080, 3058 (NH Ar); 2989-2937(CH),1575(C=S);1500(C=N); 1444(N-N);940 (C-Cl); 756, 790 (Substituted Ar). ^1H NMR (DMSO-d6, 300 MHz, ppm): 1.2 (q, 8H, CH_2), 1.5(t, 4H, CH_2); 1.6(s, NH); 7.0 (s, NH); 7.4-7.5 (Ar-H). ^{13}C NMR (DMSO-d6, 75.4 MHz, ppm, DEPT): 188.25 (C=S);156.12(C=N);135.88 (ar-C-N); 131.28 (Ar- C-Cl); 130.06-109.94 (Ar-4C); 77.32, 77.00, 76.69,42.31, 37.98, 32.12(CH_2). Mass m/z:296 (M+). Anal.Calcd. For C_{14} H_{18} N_3 S Cl (295.83): Calcd.: C 56.84, H- 6.13, N-14.20, S-10.84; Found:C 49.61, H-4.25, N-16.83, S- 12.48

Synthesis of N-(substituted -benzylidine amine)-3-cycloalkylidine-thiosemicarbazide (4-27)

A mixture of cycloalkylidinethiosemicarbazide (0.01mol) and an aromatic aldehyde (0.01 mol) and methanol (20 mL) with few drops of glacial acetic acid was refluxed for 3-4 hrs. The reaction mixture was then cooled to room temperature and kept overnight. The separated solid was filtered out, washed with methanol dried and crystallized with mixture of ethanol and acetic acid to obtain pure compound [29].

N-(3-nitrobenzylidineamine)-3-cyclopentylidine-thiosemicarbazide (4)

IR ν cm^{-1}: 3490 (NH); 3158-3090 (Ar-CH); 2809-2975 (CH); 1602 (C=N); 1068 (C=S); 1349, 1535 (N-O); 1472 (N-N); 937(C-N); 703, 844 (m-substitution at Ar ring). ^1H NMR (DMSO-d6, 300 MHz, ppm): 2.5 (q, 4H,CH_2); 3.3(t, 4H,CH_2); 7.6(s, N=CH); 8.1-8.3(m, Ar-H); 11.67 (s, NH). ^{13}C NMR (DMSO-d6, 75.4 MHz, ppm, DEPT): 178.32(C=S); 148.41(C=N);137.94 (=CH); 139.93, 136.22, 133.20, 130.16, 123.98, 121.42 (Ar-C(6); 40.34, 40.07, 39.79, 39.51, 39.23(CH_2). Mass m/z: 291(M+). Anal.Calcd. For $C_{13}H_{14}N_4O_2S$ (290.34): Calcd.:C, 53.78; H, 4.86; N, 19.30; O,11.02; S, 11.04; Found: C, 53.92; H, 4.61ee; N, 19.17; O,11.23 ; S, 11.2.

N-(4-chlorobenzylidine amine)-3-cyclopentylidine-thiosemicarbazide (5)

IR ν cm^{-1}: 3480 (NH); 3166-3023(Ar-CH),;2976-2871 (CH); 1600(C=N); 1368(C-N-C); 1091 (C=S); 1017(C-Cl); 830-946 (Substituted Ar). ^1H NMR (DMSO-d6, 300 MHz, ppm): 1.2 (q, 8H, CH_2), 1.5(t, 4H, CH_2); 1.6(s, NH); 7.0 (s, NH); 7.4-7.5 (Ar-H). ^{13}C NMR (DMSO-d6, 75.4 MHz, ppm, DEPT): 178.06(C=S); 140.58(C=N);134.94(C-Cl); 133.70 -128.75 (Ar-C); 40.07, 39.79, 39.51, 39.23(CH_2). Mass m/z: 280(M+). Anal.Calcd. ForC_{13} H_{14} N_3 S Cl (279.79): Calcd.:C, 55.81; H, 5.04; N, 15.02; S, 11.46; Found: C, 55.97; H, 5.25; N, 15.46; S, 11.83.

N-(2-nitrobenzylidineamine)-3-cyclopentylidine-thiosemicarbazide (6)

IR ν cm^{-1}: 3468 (NH); 3108-3023(Ar-CH); 2955-2976 (=CH); 1601 (C=N); 1058 (C=S); 1332, 1527 (N-O); 1472 (N-N); 1362(C-N-C); 915(C-N for nitro Ar); 709-856 (Substituted Ar). ^1H NMR (DMSO-d6, 300 MHz, ppm): 1.2(q, 4H, CH_2); 2.5 (t, CH_2); 3.3(t, CH_2); 7.5(s, N=CH); 8.1-8.3(m, Ar-H); 11.74 (s, NH). ^{13}C NMR (DMSO-d6, 75.4 MHz, ppm, DEPT): 178.45(C=S); 148.29(C=N); 137.18-124.53 (Ar-C(6); 48.43, 40.33, 39.50, 38.66 (CH_2). Mass m/z: 391(M+). Anal.Calcd. For C_{13} H_{14} N_4 S O_2 (290.34): Calcd.: C, 53.78; H,4.86; N, 19.30; O, 11.02; S,11.04; Found:C, 53.51; H,4.62; N, 19.37; O, 11.23; S,11.18.

N-(2-hydroxybenzylidineamine)-3-cyclopentylidine-thiosemicarbazide (7)

IR ν cm^{-1}: 3550(C-OH); 3456 (NH); 3110-3022(Ar-CH); 2959-2981 (=CH); 1604 (C=N); 1056 (C=S); 1343, 1557 (N-O); 1472 (N-N), 917(C-N for nitro Ar); 705-846 (Substituted Ar). ^1H NMR (DMSO-d6, 300 MHz, ppm): 1.2(m, 2H, CH_2); 2.5 (m, 2H, CH_2); 3.3(t, 4H, CH_2); 6.7(s, N=CH);7.5-8.3(m, Ar-H); 9.8 (s, OH); 11.22 (s, NH). ^{13}C NMR (DMSO-d6, 75.4 MHz, ppm, DEPT): 177.44(C=S); 159.22(Ar-C-OH); 142.67(C=N); 129.00-115.5 (Ar-4C), 40.35, 39.79, 39.24, 38.96 (CH_2). Mass m/z: 262 (M+). Anal.Calcd. For C_{13} H_{15} N_3 S O(261.34): Calcd.: C, 59.74; H, 5.79; N, 16.08; O, 6.12; S, 12.27; Found: C, 59.57; H, 5.45; N, 16.21; O, 6.24; S, 12.31.

N-(Imidazolidineamine)-3-cyclopentylidine-thiosemicarbazide(8)

IR ν cm^{-1}: 3230-3134 (NH); 3010-2994(Ar-CH); 2876-2957 (CH); 1586, 1868 (C=N); 1103 (C=S); 1472 (N-N); 1301(C-N-C). ^1H NMR (DMSO-d6, 300 MHz, ppm): 1.7(t, 2H, CH_2); 1.9 (t, 2H, CH_2); 2.5 (t, 4H, CH_2); 7.1(s, N=CH);7.8-8.3(m, Ar-2H);12.49 (s, NH), (9.8 (s, NH), 11.55 (s, NH). ^{13}C NMR (DMSO-d6, 75.4 MHz, ppm, DEPT): 178.42(C=S); 155.67(C=N); 146.32(C=N); 132.23-119.24(hetcyc-C (3); 39.35, 29.73, 26.34, 26.56(CH_2). Mass m/z: 236 (M+). Anal.Calcd. ForC_{10} H_{13} N_5 S (235.31): Calcd.: C, 51.04; H, 5.57; N, 29.76; S, 13.63; Found: C, 51.32; H, 5.45; N, 29.41; S, 13.90.

N-(4-Methyl-thiazol-2-ylmethylene)-3-cyclopentylidine-thiosemicarbazides(9)

IR ν cm^{-1}: 3454(NH); 3148-3077(Ar-CH); 2976-2831(CH); 1602 (C=N); 1110(C=S); 1457 (N-N); 1338 (C-N-C); 705-838 (Substituted Ar).^1H NMR (DMSO-d6, 300 MHz, ppm): 1.9(s, 3H, CH_3); 2.3-2.5 (t, 4H, CH_2); 3.3 (t, 4H, CH_2); 7.3(s, N=CH),8.2(Ar-2H);11.68 (s, NH). ^{13}C NMR (DMSO-d6, 75.4 MHz, ppm, DEPT): 178.10(C=S); 162.82(C=N); 153.29(C=N); 136.89(2**C**-hetcyc); 116.31(CH_3-C-hetcy) 40.36, 39.80, 39.25, 38.97(CH_2); 16.60(CH_3). Mass m/z: 267 (M+). Anal.Calcd. For C_{11} H_{14} N_4 S_2 (266.39): Calcd.: C, 51.04; H, 5.57; N, 29.76; S, 13.63; Found:C, 51.23; H, 5.69; N, 29.65; S, 13.79.

N-(4-dimethyl-benzylamine)-3-cyclopentylidenethiosemicarbazides (10)

IR ν cm^{-1}: 3453(NH); 3110, 3011(Ar-CH); 2901-2884(CH); 1597 (C=N); 1148(C=S); 1459 (N-N); 1336(C-N-C); 1283 (C-N-dimethamine); 810, 875 (Substituted Ar). ^1H NMR (DMSO-d6, 300 MHz, ppm): 2.5(q, 4H, CH_2); 2.9 (s, 6H); 3.3 (t, 4H, CH_2); 6.6(s, N=CH);7.5-7.9(Ar-6H); 11.15 (s, NH). ^{13}C NMR (DMSO-d6, 75.4 MHz, ppm, DEPT): 2.5(q, 4H, CH_2); 2.9 (s, 6H); 3.3 (t, 4H, CH_2); 6.6(s, N=CH);7.5-7.9(Ar-6H); 11.15 (s, NH). Mass m/z: 289 (M+). Anal.Calcd. For C_{15} H_{20} N_4 S (288.41): Calcd.: C, 62.47; H, 6.99; N, 19.43; S, 11.12; Found:C, 62.42; H, 6.52; N, 19.37; S, 11.28.

N-(2-chlorobenzylidine amine)-3-cyclopentylidine-thiosemicarbazide (11)

IR ν cm^{-1}: 3430 (NH); 3155-3112(Ar-CH); 2983-2793 (CH); 1611(C=N); 1374(C-N-C); 1103 (C=S); 1047(C-Cl); 821-869(Substituted Ar). ^1H NMR (DMSO-d6, 300 MHz, ppm): 2.5 (m, 4H, CH_2); 3.1-3.3(t, 4H,CH_2); 7.3(s, N=CH);8.4- 8.1(m, Ar-H); 11.63 (s, NH). ^{13}C NMR (DMSO-d6, 75.4 MHz, ppm, DEPT): 178.22(C=S); 138.15(C=N);133.12(N=CH); 131.49-127.35(Ar-6C); 40.35, 39.80, 39.24, 38.96 (CH_2). Mass m/z: 280 (M+). Anal.Calcd. ForC_{13} $H_{14}ClN_3$ S (279.79): Calcd.: C, 55.81; H, 5.04; N, 15.02; S, 11.46; Found:C, 55.54; H, 5.12; N, 15.33; S, 11.32.

N-(3-nitrobenzylidineamine)-3-cyclohexylidine-thiosemicarbazide (12)

IR ν cm^{-1}: 3397 (NH); 3160-3094(Ar-CH); 2812-2986 (CH); 1603 (C=N); 1069 (C=S); 1348 (C-N-C); 1537 (N-O); 1471 (N-N); 937(C-N); 704, 844 (Substituted Ar). ^1H NMR (DMSO-d6, 300 MHz, ppm): 1.2-1.3 (t, 2H, CH_2); 2.5 (q, 4H, CH_2); 3.3(t, 4H, CH_2); 7.6(s, N=CH); 8.1-8.6(m, Ar-H); 11.61 (s, NH). ^{13}C NMR (DMSO-d6, 75.4 MHz, ppm, DEPT): 178.33(C=S); 148.38(C=N);139.89 (N=CH); 136.18, 133.52, 130.10, 123.91, 121.36 (Ar-6C); 40.34, 40.06, 39.79, 39.51, 39.23, (CH_2). Mass m/z: 305 (M+). Anal.Calcd. For C_{14} H_{16} N_4O_2 S (304.37): Calcd.: C, 55.25; H, 5.30; N, 18.41; S, 10.53; O, 10.51; Found: C, 55.67; H, 5.56; N, 18.34; S, 10.89; O, 10.32.

N-(4-chlorobenzylidine amine)-3-cyclohexylidenethiosemicarbazide (13)

IR ν cm^{-1}: 3438 (NH); 3165-3025(Ar-CH); 2994-2887 (CH); 1600(C=N); 1490 (N-N); 1091 (C=S); 1367(C-N-C); 1017(C-Cl); 700-830 (Substituted Ar). ^1H NMR (DMSO-d6, 300 MHz, ppm): 1.2-1.3 9t, 2H, CH_2); 2.5 (q, 4H, CH_2); 3.1-3.3(t, 4H,CH_2); 7.4(s, N=CH); 7.7-8.7(m, Ar-H); 11.48 (s, NH).^{13}C NMR (DMSO-d6, 75.4 MHz, ppm, DEPT): 178.07(C=S); 134.21 (C=N);140.81 (N=CH); 133.19(Ar-C-Cl); 128.94-128.68(Ar-6C); 40.34, 39.78, 39.51, 39.23, 38.95 (CH_2). Mass m/z: 294 (M+). Anal.Calcd. For C_{14} H_{16} Cl N_3 S (293.81): Calcd.: C, 57.23; H, 5.49; N, 14.30; S, 10.91; Found:C, 57.57; H, 5.25; N, 14.46; S, 10.83.

N-(2-nitrobenzylidineamine)-3-cyclohexylideneylidinethiosemicarbazide (14)

IR ν cm^{-1}: 3450 (NH); 3151-3083(Ar-CH); 2975-2800 (CH); 1601 (C=N); 1125 (C=S); 1333, 1539 (N-O); 1472 (N-N); 1311(C-N-C); 916(C-N); 705-850 (Substituted Ar). ^1H NMR (DMSO-d6, 300 MHz, ppm): 1.2(t, 2H, CH_2); 2.5-2.6 (m, 4H,CH_2); 2.9-3.3(t, 4H, CH_2); 7.4(s, N=CH); 7.7-8.7(m, Ar-H); 11.742(s, NH). ^{13}C NMR (DMSO-d6, 75.4 MHz, ppm, DEPT): 178.49(C=S); 148.29(HC=N); 137.22 (C=N); 133.34(C-NO2); 130.36-124.50 (Ar-5C); 40.35, 40.07, 39.79, 38.96, 38.68 (CH_2). Mass m/z: 305 (M+). Anal.Calcd. For C_{14} H_{16} N_4O_2 S (304.37): Calcd.: C, 55.25; H, 5.30; N, 18.41; O, 10.51; S, 10.53; Found:C, 55.45; H, 5.49; N, 18.98; O, 10.12; S, 10.29.

N-(2-hydroxybenzylidineamine)-3-cyclohexylidenethiosemicarbazide (15)

IR ν cm^{-1}: 3578 (OH); 3456 (NH); 3151-3014(Ar-CH); 2808-2981 (CH); 1598 (C=N); 1056 (C=S); 1376, 1554 (N-O); 1461(N-N);1305 (C-N-C); 917(C-N); 904 -947(Substituted Ar). ^1H NMR (DMSO-d6, 300 MHz, ppm): 1.3 (t, 2H, CH2); 2.5 (q, 4H, CH_2); 3.3(t, 4H); 6.7(s, N=CH); 7.5-8.0(m, Ar-H); 9.8 (s, OH); 11.26 (s, NH). ^{13}C NMR (DMSO-d6, 75.4 MHz, ppm, DEPT): 177.42(C=S); 159.27(Ar-C-OH); 142.71(HC=N); 129.09(C=N); 125-115.5 (Ar-C); 40.34, 39.79, 39.51, 38.96, 38.68 (CH_2). Mass m/z: 276 (M+).Anal.Calcd. For C_{14} H_{17} N_3 SO (275.37): Calcd.: C, 61.06; H, 6.22; N, 15.26; O, 5.81; S, 11.64; Found:C, 61.23; H, 6.16; N, 15.51; O, 5.78; S, 11.41.

N-(imidazolidineamine)-3-cyclopentylidine-thiosemicarbazide (16)

IR ν cm^{-1}: 3460, 3271 (NH); 3085, 3046(Ar-CH); 2928 (CH); 1623, 1594 (C=N); 1089 (C=S); 1456 (N-N); 1306(C-N-C); 793, 847 (Substituted Ar). ^1H NMR (DMSO-d6, 300 MHz, ppm): 1.6(t, 2H, CH_2); 2.5(q, 4H, CH_2); 3.3(t, 4H, CH_2); 7.0(s, N=CH); 7.0-8.3(m, Ar-

H); 11.57(s, NH); 12.50 (s, Cyclic NH). ^{13}C NMR (DMSO-d6, 75.4 MHz, ppm, DEPT): 177.23(C=S), 154.56(C=N), 145.34(C=N), 131.23-118.23(hetcyc-C(3), 38.35, 34.73, 29.34, 28.23, 26.56 (CH_2). Mass m/z: 250 (M+). Anal.Calcd. For $C_{11}H_{15}N_5S$ (249.34): Calcd.: C, 52.99; H, 6.06; N, 28.09; S, 12.86; Found: C, 52.87; H, 6.26; N, 28.19; S, 12.67.

N-(4-Methyl-thiazol-2-ylmethylene)-3-cyclohexylidenethiosemicarbazides(17)

IR ν cm^{-1}: 3445(NH); 3224-48-3078(hetcyc-CH); 2981-2857(CH); 1603, 1592 (C=N); 1112(C=S); 1457 (N-N); 1336(C-N-C); 764, 838 (Substituted Ar). ^1H NMR (DMSO-d6, 300 MHz, ppm): 1.9(s, 3H, CH_3); 2.3 (t, 2H, CH_2); 2.5 (q, 4H, CH_2); 3.3 (t, 4H, CH_2); 7.3(s, N=CH);8.4-8.2(s, Hetcyc-H); 11.70 (s, NH). ^{13}C NMR (DMSO-d6, 75.4 MHz, ppm, DEPT): 178.07(C=S); 162.87(C=N); 153.32(C=N); 136.92(2**C**-hetcyc); 116.31(C-hetcy); 40.35, 39.79, 39.23, 38.96,38.68(CH_2); 16.66(CH_3). Mass m/z: 281 (M+). Anal.Calcd. For $C_{12}H_{16}$ N_4 S_2 (280.41): Calcd.: C, 51.40; H, 5.75; N, 19.98; S, 22.87; Found: C, 51.67; H, 5.89; N, 19.76; S, 22.97.

N-(4-dimethyl-benzylamine)-3-cyclohexylidenethiosemicarbazides (18)

IR ν cm^{-1}: 3459(NH); 3138(Ar-CH); 2989-2854(CH); 1595, 1587 (C=N); 1126(C=S); 1464 (N-N); 1339(C-N-C); 1278 (C-N, dimethyl amine); 820, 878 (Substituted Ar). ^1H NMR (DMSO-d6, 300 MHz, ppm): 2.5(t, 2H, CH_2); 2.9 (s, 6H, CH_3); 3.1(q, 4H, CH_2), 3.3 (t, 4H, CH_2); 6.6(s, N=CH);7.5-8.0(Ar-6H);11.18 (s, NH). ^{13}C NMR (DMSO-d6, 75.4 MHz, ppm, DEPT): 176.99(C=S); 151.38(C=N); 143.32(C=N); 128.62-111.69(Ar-6C); 47.15(CH_3), 40.35, 40.07, 39.79, 39.51, 39.24(CH_2). Mass m/z: 303 (M+). Anal.Calcd. For C_{16} $H_{22}N_4S$ (302.44): Calcd.: C, 63.54; H, 7.33; N, 18.53; S, 10.60; Found:C, 63.87; H, 7.53; N, 18.51; S, 10.32.

N-(2-chlorobenzylidine amine)-3-cyclopentylidine-thiosemicarbazide (19)

IR ν cm^{-1}: 3340 (NH); 3179-3125(Ar-CH); 2983-3022 (CH); 1610, 1594(C=N); 1375(C-N-C); 1103 (C=S); 1046(C-Cl); 710-869(Substituted Ar). ^1H NMR (DMSO-d6, 300 MHz, ppm): 1.9(q, 2H, CH_2); 2.5 (q, 4H, CH_2); 3.3(t, 4H,CH_2); 7.3(s, N=CH);8.4- 8.1(m, Ar-H); 11.65 (s, NH). ^{13}C NMR (DMSO-d6, 75.4 MHz, ppm, DEPT): 178.21(C=S); 138.15(HC=N);133.13(N=C); 131.49-127.35(Ar-6C); 40.35, 39.79, 39.51, 38.68, 35.21(CH_2). Mass m/z: 294 (M+). Anal.Calcd. For C_{14} H_{16} Cl N_3 S (293.91): Calcd.: C, 57.23; H, 5.49; N, 14.30; S, 10.19; Found:C, 57.67; H, 5.82; N, 14.43; S, 10.21.

N-(3-nitrobenzylidineamine)-3-cyclohepylidenethiosemicarbazide (20)

IR ν cm^{-1}: 3383, 3238 (NH); 3192-3091(Ar-CH); 2810-2987 (CH); 1603 (C=N); 1069 (C=S); 1349 (C-N-C); 1528 (N-O); 1471 (N-N); 937(C-N nitroaromatic compounds); 703, 820 (Substituted Ar). ^1H NMR (DMSO-d6, 300 MHz, ppm): 2.5 (m, 6H, CH_2); 3.3(t, 4H, CH_2); 7.6(s, N=CH); 8.1-8.6(m, Ar-H); 11.59 (s, NH). ^{13}C NMR (DMSO-d6, 75.4 MHz, ppm, DEPT): 178.36(C=S); 148.39(HC=N); 139.91 (N=C); 136.18, 133.50, 130.10, 123.91, 121.36 (Ar-6C); 40.35, 40.07, 39.79, 39.51, 39.24, 38.96(CH_2). Mass m/z: 319 (M+). Anal.Calcd. For C_{15} H_{18} N_4 O_2 S (318.39): Calcd.: C, 56.58; H,5.70; N,17.60; S,10.05; O,10.07; Found:C, 56.25; H,5.87; N,17.34; S,10.14; O,10.32.

N-(4-chlorobenzylidine amine)-3-cycloheptylidenethiosemicarbazide (21)

IR ν cm^{-1}: 3423, 3273(NH); 3158-3056(Ar-CH); 2995-2772 (CH); 1610(C=N); 1490 (N-N); 1091 (C=S); 1367(C-N-C); 1017(C-Cl); 700-830 (Substituted Ar). ^1H NMR (DMSO-d6, 300 MHz, ppm): 2.5 (q, 6H, CH_2); 3.3(t, 4H, CH2); 7.4(s, N=CH); 8.2-7.8(m, Ar-H); 11.49 (s, NH). ^{13}C NMR (DMSO-d6, 75.4 MHz, ppm, DEPT): 178.07(C=S); 134.26 (C=N);140.86 (N=CH); 133.23(Ar-C-Cl); 128.99-128.73(Ar-6C); 40.34, 39.79, 39.51, 39.23, 38.95,38.67(CH_2). Mass m/z: 308 (M+). Anal.Calcd. For C_{15} H_{18}Cl N_3 S (307.84): Calcd.: C, 58.52; H, 5.89; N, 13.65; S, 10.42; Found: C, 58.76; H, 5.97; N, 13.32; S, 10.30.

N-(2-nitrobenzylidineamine)-3-cycloheptylidenethiosemicarbazide (22)

IR ν cm^{-1}: 3458 (NH); 3228-3107 (Ar-CH); 3083-2974 (CH); 1601 (C=N); 1105 (C=S); 1334, 1541 (N-O); 1471 (N-N); 1311(C-N-C); 930(C-N strvib for nitroaromatic compounds); 722-849 (Substituted Ar). ^1H NMR (DMSO-d6, 300 MHz, ppcm): 2.5 (m, 8H, CH_2); 3.3(t, 4H, CH_2); 7.6(s, N=CH); 7.7-8.4(m, Ar-H); 11.73(s, NH). ^{13}C NMR (DMSO-d6, 75.4 MHz, ppm, DEPT): 178.47(C=S); 148.30(HC=N); 137.21(C=N); 133.36(C-NO2); 130.36-124.53 (Ar-5C); 40.34, 40.07, 39.79, 39.51, 38.96, 38.68 (CH_2). Mass m/z: 319 (M+). Anal.Calcd. For C_{15} H_{18} N_4O_2 S (318.39): Calcd.: C, 56.58,;H, 70.01; N,17.60; O, 10.05; S, 10.07; Found: C, 56.39,;H, 70.12; N,17.69; O, 10.06; S, 10.13.

N-(2-hydroxybenzylidineamine)-3-cycloheptylidenethiosemicarbazide (23)

IR ν cm^{-1}: 3580 (OH); 3457(NH); 3127-3003(Ar-CH); 2806-2948 (CH); 1608, 1585 (C=N); 1097 (C=S); 1374, 1547 (N-O); 1445(N-N); 1307(C-N-C); 822, 834 (Substituted Ar). ^1H NMR (DMSO-d6, 300 MHz, ppm): 2.5 (m, 8H, CH_2); 3.1-3.3(t, 4H, CH_2); 6.7(s, N=CH); 7.5-8.0(m, Ar-H); 9.84 (s, OH); 11.22 (s, NH). ^{13}C NMR (DMSO-d6, 75.4 MHz, ppm, DEPT): 177.44(C=S); 159.22(Ar-C-OH); 142.68(HC=N); 129.01(C=N); 125-115.51 (Ar-C); 40.34, 39.78, 39.51, 39.23, 38.95, 38.67(CH_2). Mass m/z:290 (M+). Anal.Calcd. For C_{15} H_{19} N_3O S (289.40): Calcd.: C, 62.25; H, 6.62; N, 14.52; O, 5.53; S,11.08; Found: C, 62.56; H, 6.78; N, 14.34; O, 5.78; S,11.10.

N-(imidazoledineamine)-3-cyclopentylidine-thiosemicarbazide (24)

IR ν cm^{-1}: 3360, 3250 (NH); 3044, 3006(Ar-CH); 2999-2803 (CH); 1588 (C=N); 1097 (C=S); 1455(N-N); 1290(C-N-C); 794, 839 (Substituted Ar). ^1H NMR (DMSO-d6, 300 MHz, ppm): 1.5-1.6(m, 4H, CH_2); 2.5(m, 4H, CH_2); 3.3(t, 4H, CH_2); 7.0(s, N=CH); 7.4-8.5(m, Ar-H); 9.7 (s, NH); 12.50 (s, Cyclic NH). ^{13}C NMR (DMSO-d6, 75.4 MHz, ppm, DEPT): 177.54(C=S); 157.23(C=N); 144.43(C=N); 133.23-114.34(hetcyc-C(3); 38.32, 34.75, 29.44, 27.16(CH_2). Mass

m/z: 264 (M+). Anal.Calcd. For C_{12} H_{17} N_5 S (263.36): Calcd.: C, 54.73; H, 6.51;N, 26.59; S, 12.18; Found: C, 54.45; H, 6.23;N, 26.66; S, 12.14.

N-(4-Methyl-thiazol-2-ylmethylene)-3-cycloheptylidenethiosemicarbazides (25)

IR ν cm^{-1}: 3490(NH); 3224-3143(hetcyc-CH); 2924-3077(CH); 1603, 1592 (C=N); 1111(C=S); 1461 (N-N); 1336(C-N-C); 764, 838 (Substituted Ar). ^1H NMR (DMSO-d6, 300 MHz, ppm): 1.9(s, 3H, CH_3); 2.3-2.5 (m, 8H, CH_2); 3.1-3.3 (t, 4H, CH_2); 7.3(s, N=CH);8.4-7.6(s, Hetcyc-H);11.72 (s, NH).^{13}C NMR (DMSO-d6, 75.4 MHz, ppm, DEPT): 178.06(C=S); 162.91(HC=N);153.34(C=N); 136.94(2C-hetcyc); 116.31(C-hetcy.CH_3); 40.34, 39.79, 39.51, 39.23, 38.95, 38.68 (CH_2); 16.66(CH_3). Mass m/z: 295 (M+). Anal.Calcd. For C_{13} H_{18} N_4 S_2 (294.44): Calcd.: C, 53.03; H, 6.16; N, 19.03; S, 21.78; Found: C, 53.23; H, 6.45; N, 19.14; S, 21.95.

N-(4-dimethyl-benzylamine)-3-cyclohexylidenethiosemicarbazides (26)

IR ν cm^{-1}: 3489(NH); 3121, 3018 (Ar-CH); 2978-2802(CH); 1590, 1578 (C=N); 1085(C=S); 1462 (N-N); 1309(C-N-C); 1248 (C-N, dimethyle amine); 810, 875 (Substituted Ar). ^1H NMR (DMSO-d6, 300 MHz, ppm): 2.5(m, 8H, CH_2); 2.9(s, 6H); 3.3 (t, 4H, CH_2); 6.6(s, N=CH);7.5-7.9(Ar-6H);11.17 (s, NH). ^{13}C NMR (DMSO-d6, 75.4 MHz, ppm, DEPT): 176.97(C=S); 151.39(C=N);143.30(C=N); 128.63-111.69(Ar-6C); 40.34(CH_3); 40.07 (CH_3); 39.79, 39.51, 39.23, 38.96, 38.68 (CH_2). Mass m/z: 317 (M+). Anal.Calcd. For C_{17} H_{24} N_4 S (316.46): Calcd.: C, 64.52; H, 7.64; N, 17.70; S, 10.13; Found: C, 64.66; H, 7.29; N, 17.89; S, 10.24.

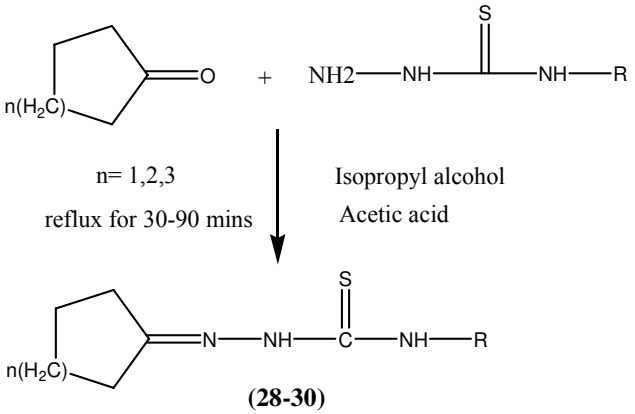
N-(2-chlorobenzylidine amine)-3-cycloheptylidenethiosemicarbazide (27)

IR ν cm^{-1}: 3440 (NH); 3240-3151(Ar-CH); 2984-3062 (CH); 1611, 1593(C=N); 1374(C-N-C); 1103 (C=S); 1047(C-Cl); 756-870(Substituted Ar). ^1H NMR (DMSO-d6, 300 MHz, ppm): 2.5 (q, 8H, CH_2); 3.1-3.3(t, 4H, CH_2); 7.3(s, N=CH);8.4- 8.1(m, Ar-H); 11.64 (s, NH). ^{13}C NMR (DMSO-d6, 75.4 MHz, ppm, DEPT): 178.21(C=S);138.14(HC=N);133.13(N=C); 131.50-127.36(Ar-6C); 40.35, 40.07, 39.79, 39.51, 38.96, 38.68(CH_2). Mass m/z: 308 (M+). Anal.Calcd. For Calcd.: C, 58.52; H, 5.89; N, 13.65; S-10.42; Found: C, 58.67; H, 5.56; N, 13.32; S-10.10.

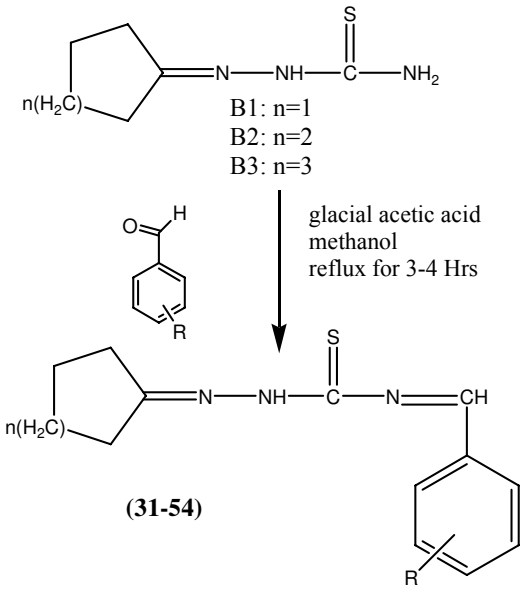
Results and discussions

Chemistry

We have utilized thiosemicarbazide and cycloalkanone as two potential synthetic blocks and reported the synthesis of twenty seven cycloalkylidenethiosemicarbazides (1-27) as depicted in Scheme 1 and scheme 2. Scheme 1 involved condensation reaction between substituted thiosemicarbazides and cycloalkanones resulted in to formation of cycloalkylidenethiosemicarbazides (1-3). Scheme 2 is characterized by reaction between terminal ^1N of cycloalkylidenethiosemicarbazides and various substituted aromatic aldehydes and resulted in to the formation of cycloalkylidenethiosemicarbazide derivatives (4-27) as described in scheme 2.



Scheme 1: Synthesis of N-(3-substituted phenyl)-3-cycloalkylidene-thiosemicarbazides

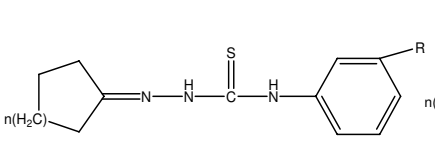


Scheme 2: Synthesis of N-(substituted -benzylidine amine)-3-cycloalkylidine-thiosemicarbazides

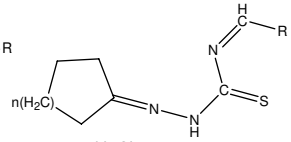
Structures of all compounds synthesized were identified by ^{1}H NMR, ^{13}C NMR, FT-IR and elemental analysis. The Infrared spectra (IR) of each of the synthesized compounds showed absorption bands which are characteristic of the anticipated structure of the synthesized compounds. Infrared measurements showed the presence of NH (3400-3600 cm^{-1}), C=N (1580-1611 cm^{-1}) and C=S (1010-1598 cm^{-1}) stretches at their expected frequencies. Moreover, the presence of NO_2 group in 4, 6, 12, 14, 20 and 22was indicated by the appearance of asymmetric and symmetric NO_2 stretching bands at 1550–1500 cm^{-1} and 1365–1335 cm^{-1}, respectively.Compounds 1, 2, 3, 5, 11, 13, 19, 21and 27 showed C–Cl stretchings at 830-1049 cm^{-1}. More significantly, a ^{13}C NMR chemical shift at 176.11-180.50 ppm was indicative of a C=S moiety. All the compounds exhibited ^{13}C NMR chemical shift for HC=N at 133.10-158.11 ppm and for C=N at 138.40-162.56 ppm. Cyclic protons appeared as multiplate or triplet between 1.2-2.5 through ^{1}H NMR. Mass spectra of compounds (1-

27) exhibited molecular ion peak at their expected m/z values. All NH protons were appeared as singlet in ^{1}H NMR at 11.2-11.5 ppm. Peaks at 7.4-8.5 in compounds 4-27 accounted for presence of aromatic groups through ^{1}H NMR which is further confirmed by the presence of peaks at 111.23-138.56 ppm in ^{13}C NMR. The ^{13}C spectrum corroborated the proton and IR data, clearly displaying the expected signals (C=S, C=O, C-O, C=N, cycloalkyl carbons (5C-7C), CH, CH_2, and CH_3). Other characterization data is cited in Table 1.

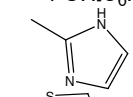
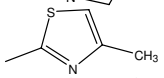
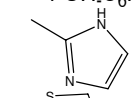
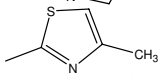
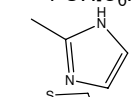
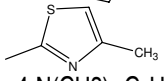
Table 1: Characterization data of compounds



(1-3)



(4-27)

Compound No.	m.p. (C)	Yield (%)	n	R
1	147-149	76	Cyclopentyl	Cl
2	123-125	66	Cyclohexyl	Cl
3	122-125	55	Cycloheptyl	Cl
4	159-161	72	Cyclopentyl	3-NO_2.C_6H_4
5	169-172	69	Cyclopentyl	4-Cl- C_6H_4
6	210-215	51	Cyclopentyl	2- NO_2.C_6H_4
7	189-190	61	Cyclopentyl	4-OH.C_6H_4
8	105-111	55	Cyclopentyl	
9	180-184	56	Cyclopentyl	
10	165-167	58	Cyclopentyl	4-N(CH3)$_2$.C_6H_4
11	174-176	65	Cyclopentyl	2-Cl- C_6H_4
12	190-192	69	Cyclohexyl	3-NO_2.C_6H_4
13	171-175	79	Cyclohexyl	4-Cl- C_6H_4
14	211-213	48	Cyclohexyl	2- NO_2.C_6H_4
15	183-185	66	Cyclohexyl	4-OH.C_6H_4
16	128-130	68	Cyclohexyl	
17	181-182	59	Cyclohexyl	
18	150-154	51	Cyclohexyl	4-N(CH3)$_2$.C_6H_4-
19	127-130	70	Cyclohexyl	2-Cl- C_6H_4
20	190-191	67	Cycloheptyl	3-NO_2.C_6H_4
21	169-173	59	Cycloheptyl	4-Cl- C_6H_4
22	208-211	59	Cycloheptyl	2- NO_2.C_6H_4
23	182-186	58	Cycloheptyl	4-OH.C_6H_4
24	81-83	72	Cycloheptyl	
25	180-182	61	Cycloheptyl	
26	159-162	74	Cycloheptyl	4-N(CH3)$_2$.C_6H_4-
27	170-172	79	Cycloheptyl	2-Cl- C_6H_4

Antimicrobial evaluation

All synthesized compounds were evaluated for antifungal and antibacterial activity using macrotube dilution method. Fluconazole and griseofulvin were used as positive control for various fungal strains and ciprofloxacin for bacterial strains for their MIC determination. Preliminary assay was performed for a total of five fungal strains and five bacterial strains (including both gram positive and gram negative strains). The included fungal isolates were *Candida albicans* (2 strains: ATCC 24433, ATCC 10231, *Candida tropicalis* ATCC 13803, *Aspergillusniger* ATCC 9029 and *Penicilliumchrysogenum* ATCC 10002. The included bacterial isolates were *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus aureus* ATCC 25923, *Serratiamarcescens* ATCC 13880, *Escherichia coli* ATCC8739.

Antifungal activity

Table 2 exhibited the antifungal activity of cycloalkylidenethiosemicarbazide derivatives (1-27) against various fungal strains. Compounds 9, 14, 24 and 25 have shown remarkable activity (16 µg/mL) against *A. niger* ATCC 9029. Compounds*6*, 8, 9, 14, 19, 21 and 25 (16 µg/mL) against *C. Albicans* ATCC 24433and compounds 14 and 27 were found to be most active against *C. Albicans* ATCC 10231(8µg/mL). Some compounds such as 6, 8, 9, 11, 18 and 25 shown remarkable activity (16 µg/mL) against *C. Albicans* ATCC 10231. Compounds 14 and 25 were found to be most active (8µg/mL) against *Candida tropicalis*ATCC 13803. Only compounds 11 and 27 have resulted in to good activity (16 µg/mL) against *Penicilliumchrysogenum* ATCC 10002. Among all cycloalkylidenethiosemicarbazide derivatives compound 14 and 27 have shown interesting activity. Compound 14 have shown more activity (8µg/mL) than standard GRS against *A. niger* ATCC 9029 and *C. albicans* ATCC 10231 and shown more activity than standard FLC against *C. tropicalis* ATCC 13803. Compound 27 exhibited more activity than GRS against *C. albicans* ATCC 10231 and *P. chrysogenum* ATCC 10002. Compound 14 was found to be most active among cycloalkylidenethiosemicarbazide derivatives against most of the fungal strains used in the study.

Table 2: Antifungal activities of cycloalkyllidenethiosemicarbazide derivatives

Compound	c log[Pa]	*Tested fungi (MIC µg/mL)				
		A	B	C	D	E
1	4.11	64	64	>64	64	>64
2	4.11	>64	64	64	64	>64
3	4.49	64	64	64	64	>64
4	3.41	64	64	64	64	>64
5	4.02	64	32	32	16	64
6	4.05	64	16	16	16	64
7	3.1	64	32	32	32	64
8	1.33	32	16	16	64	64
9	2.46	16	16	16	16	64
10	3.4	64	64	32	16	>64
11	4.02	64	16	16	16	16
12	3.73	64	64	64	64	64
13	4.33	>64	32	32	16	64
14	3.72	16	16	8	8	64
15	3.1	64	64	64	64	64
16	1.65	32	32	32	64	64
17	2.78	64	64	64	64	64
18	3.72	64	32	16	32	>64
19	4.33	32	16	32	64	64
20	4.04	64	64	64	64	64
21	4.65	32	16	32	32	64
22	4.04	64	64	64	64	>64
23	3.74	64	32	32	32	64
24	1.97	16	64	64	64	>64
25	3.1	16	16	16	8	64
26	4.04	64	64	64	64	>64

Structure activity relationship

Results of the antifungal activity (Table 2) of cycloalkylidenethiosemicarbazides indicated that all the compounds with more electron withdrawing –Cl substitution at para position (5, 13, 21) and ortho position (11, 19 and 27) of the benzene rings have shown good activity against candida strains but when we changed the substitution from chloro to nitro (comparatively less electron withdrawing) at ortho position (6, 14) the activity increased further (except in case of cycloheptyl derivatives, 22). When we introduced electron donating groups e. g. –OH, -N $(CH_3)_2$ in compounds 12, 15, 18 and 26 resulted in less activity. The presence of heterocyclic nucleus such as imidazoles and thiazoles in place of phenyl ring did not show any significant change in the activity. Phenyl ring with 3-NO_2 substituent as electron withdrawing groups (4, 12, and 20) shown very less activity against fungal strains indicated that electron withdrawing groups at meta position of the ring is not suitable for the activity (Fig. 1). Cycloalkylidene moiety shown more activity than thiourea moiety (1, 2, and 3) of thiosemicarbazides.

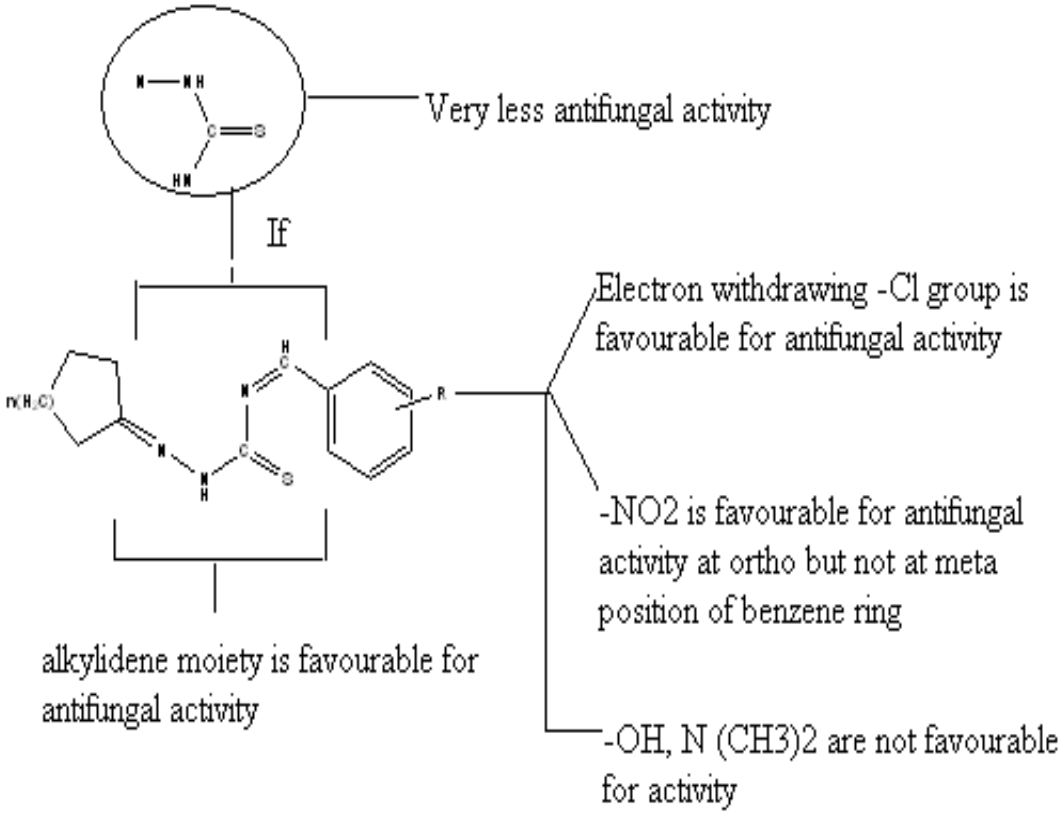


Figure 1:SAR of antifungal activity of cycloalkylidenethiosemicarbazide derivatives

Antibacterial activity

All of the cycloalkylidenethiosemicarbazide derivatives were also tested against various bacterial strains. The data reported in Table 3 indicated that compound 25 was found to be active (32 µg/mL) against *Pseudomonasaeruginosa* ATCC9027. Compound 11 have shown moderate activity (32 µg/mL) against *Staphylococcus aureus* ATCC 25923. Some compounds 1 and 18 have shown good activity against *Escherichia coli* ATCC8739. All other compounds have shown antibacterial activity less than the reference drug. In general the compounds showed low antibacterial activity as compared to antifungal activity.

Table 3: Antibacterial activities of cycloalkylidenethiosemicarbazide derivatives

Compound	*Tested bacterial strains (MIC µg/mL)				
	A	B	C	D	E
1	64	>64	>64	64	32
2	64	>64	64	64	>64
3	>64	>64	64	64	64
4	>64	>64	64	>64	64
5	>64	64	64	64	64
6	>64	64	64	>64	64
7	>64	>64	>64	>64	>64
8	>64	>64	>64	>64	>64
9	>64	>64	>64	>64	>64
10	>64	>64	>64	>64	>64
11	64	64	32	64	64
12	>64	>64	>64	>64	>64
13	>64	>64	>64	>64	>64
14	>64	64	64	64	64
15	64	64	64	64	64
16	64	>64	>64	>64	64
17	>64	64	64	64	>64
18	64	64	64	>64	32
19	64	>64	>64	>64	>64
20	64	>64	>64	>64	>64
21	64	64	64	>64	>64
22	64	64	64	64	>64
23	>64	>64	>64	>64	>64
24	>64	64	64	64	64
25	32	64	64	>64	64
26	>64	>64	64	64	>64
27	>64	>64	>64	>64	>64
Ciprofloxacin	8	2	2	8	16

Molecularmodeling

We constructed a three-dimensional model of CYP51 from *A. niger* and investigated the active site of CYP51 through homology modeling**.** Sequence of AFCYP51 protein retrieved from NCBI and shown in Fig.2. X-ray crystal structure of Human Lanosteraol 14 -demethylase (PDB Code: 3LD6) shown 38% identity with our target protein (AFCYP51), Fig. 3.

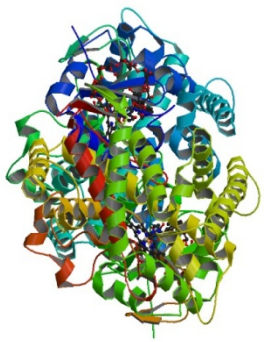


Figure 2: Crystal structure of human lanosterol 14 -demethylase (Cyp51) in complex with ketoconazole (PDB: 3LD6)

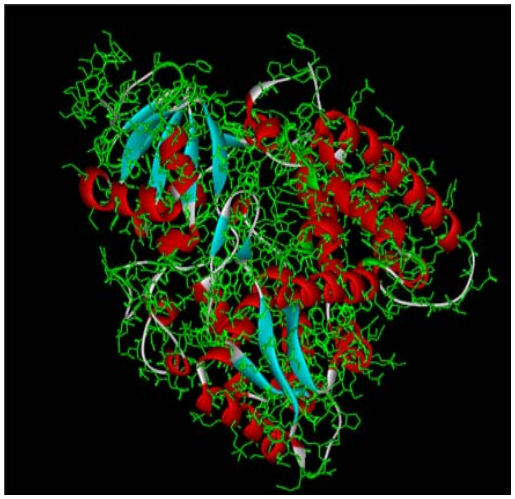


Figure 3:3D View of template in Accelerys DS Viewpro

Structure validation of protein structure modelled is processed using PROCHECK which determined stereochemical aspects along with main chain and side chain parameters with comprehensive

analysis. PROCHECK analysis revealed in Ramachandran plot (Fig. 4) concluding Phi and psi angles to contribute in conformations of amino acids excluding glycine and proline with 89.3 % (351) residues in most favoured region, 9.4%(34) in additional allowed region, 1.0%(4) in generously allowed region and 0.3%(1) residues in disallowed region.

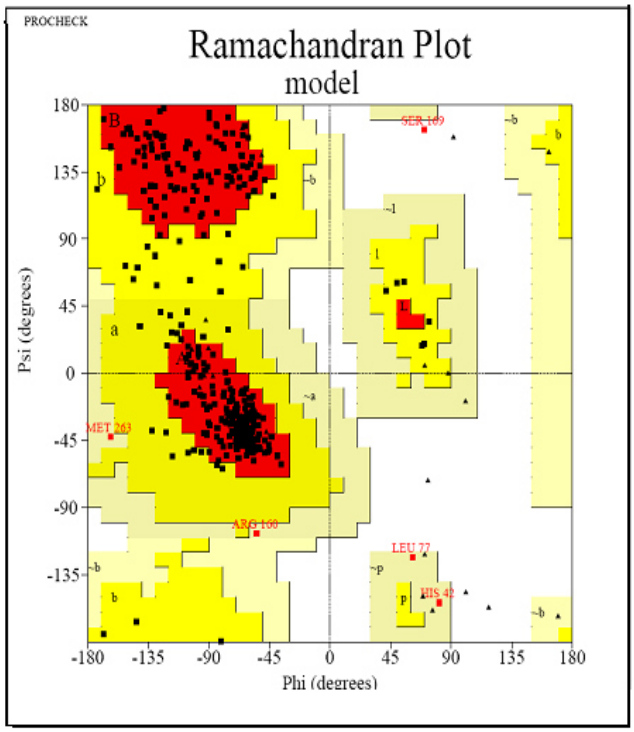


Figure 4: Ramchandran plot of homology-modeledCyp51, derived by using Deep View/Swiss-PDBViewe

The generated homology structure is validated using standard drug FLC. The binding model obtained was fully comparable to the bioactive conformation of FLC into the crystallized complex, thus demonstrating the reliability of the selected computational methodology of generating 3D structure of AFCYP51. Docking of FLC complex as standard from PDB, reported to have good binding capacity with docking score 118(min) to 125.90(max) for five poses accompanying acceptable RMSD issues from 0.831(min) to 5.8260(Max). FLC when docked in newly modeled structure of AFCYP51, active site used for all 14 candidate even yielded more interesting results with docking score 109(min) to 132(max) supported by RMSD values 1.7(min) to 9.1(max) for same five poses (Fig. 5). Protein sequence obtained from AFCYP51 falls in same position and region as described in literature. Amino acids found in the active site of 3D structure of AFCYP51were Met129, Glu130, Phe134, Ile135, Leu290, Met292, Ala293, Glu456, Cys454 and Glu131 (Fig. 6) yielded a cavity of volume 590 A and all amino acids found surrounding the corresponding positions in active site

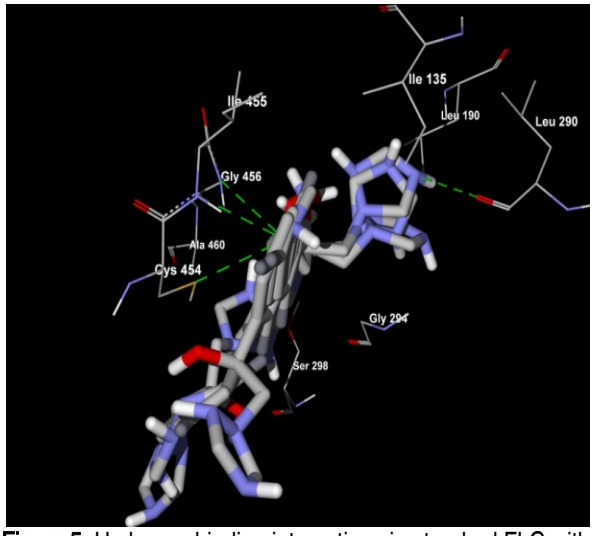


Figure 5: Hydrogen binding interactions in standard FLC with homology modelled structure of Protein CYP51, green lines indicate the hydrogen bonds

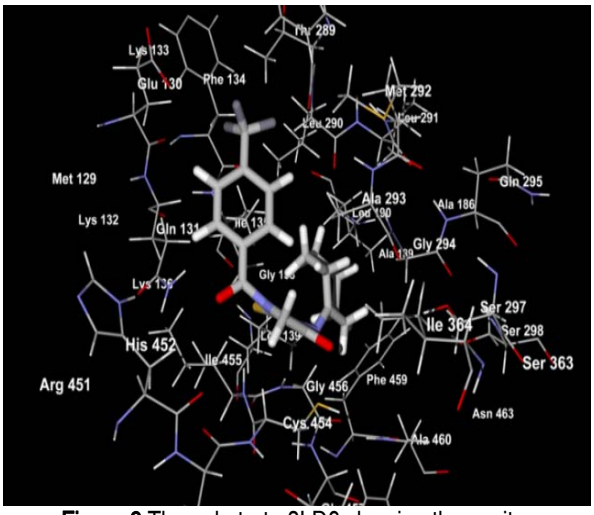


Figure 6: The substrate 3LD6 showing the cavity

Structural model of selected 14 molecules were docked on 3D model of AFCYP51. The values of RMSD obtained in results should not exceed 1.000 (according to reference ligand). When reference ligand is absent. It may be considered up to 2.0.Compound 11, 18 and 25 have shown best RMSD value of 0.944, 0.725 and 0.812 respectively and score of -83.966, -95.088 and -92.436 and shown poor hydrogen bonding with the target protein. Compound 11 and 14shown reasonable RMSD and Scores (Table 4), along with hydrogen bonding (Fig. 7). Molecular interpretation approves that C=S (green coloured atoms in Fig. 7) group present in compound 11and other similar inhibitors are critical for their antifungal activity. It contributes to most of the hydrogen bonding interactions in drug-receptor binding.

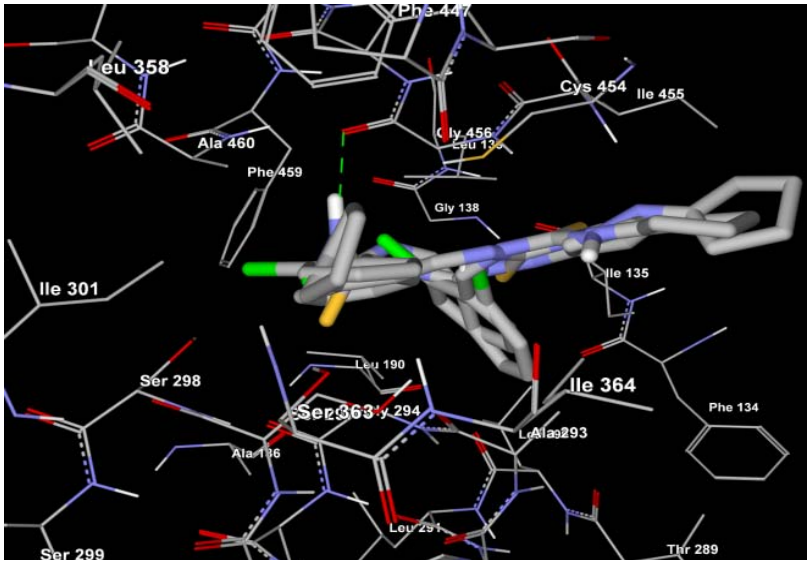


Figure 7: Hydrogen bond interactions of compound 11

Table 4: Docking scores of some cycloalkylidenethiosemicarbazide derivatives

Compounds	Best Pose	MolDock Score GRID	MolDock score	Rerank score	R.M.S.D	Torsions
5	4	-91.499	-92.533	-72.420	1.001	4
6	3	-97.134	-85.162	-67.489	3.541	5
8	3	-94.775	-92.172	-68.052	2.304	4
9	4	-100.273	-100.175	-72.950	4.975	4
10	2	-94.690	-97.864	-72.953	4.960	5
11	4	-83.966	-89.567	-71.600	0.944	4
13	4	-90.456	-91.893	-69.818	6.086	4
14	4	-87.899	-82.016	-64.612	1.723	5
18	3	-95.088	-94.603	-64.205	0.735	5
19	3	-90.313	-88.501	-73.870	1.451	5
21	1	-93.680	-94.455	-68.334	0.012	4
24	4	-89.208	-88.455	-69.965	4.228	4
25	5	-92.436	-95.056	-74.937	0.812	4
27	1	-84.963	-85.809	-71.998	1.638	4

Conclusion

A series of cycloalkylidenethiosemicarbazide derivatives were synthesized and screened for their antifungal activities using macro tube dilution method. Compound 14 with NO_2 group and compound 27 as ^{1}N-substitution to thiosemicarbazides was found to be most active against most of the fungal strains used in the study and compound 11 & 25 shown moderate activities against fungal strains. Docking studies showed that compound 25 possessed good binding scores for fungal CYP51 in the sterol biosynthesis pathway. The structure–activity relationships discussed above may provide useful information for further design and synthesis of compounds with optimized bioactivity profile. Additional studies to optimize the structural feature as well as to explore the mechanism of action and toxicity of this class of compounds are required which could be helpful in designing more potent antifungal agents for therapeutic use.

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