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Microwave Irradiated Synthesis of (E) -2-((5-(6-Methyl-2-Oxo-4-Substituted-1,2,3,4-Tetrahydropyrimidin-5-Yl) -1,3,4-Oxadiazol-2-Yl) Diazenyl) Malononitrile as Antimicrobial and Antitubercular Agents

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ABSTRACT

A new series of (E) -2-((5-(6-methyl-2-Oxo-4-substituted-1,2,3,4-tetrahydropyrimidin-5-yl) -1,3,4-oxadiazol-2-yl) diazenyl) malononitrile **(55-67)** have been synthesized by using microwave irradiation. The salient features of microwave method are rapid reaction rate, cleaner reaction condition and enhancement in chemical yield compared to conventional method. Structures of the newly synthesized compounds were assigned based on elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectral studies. The antimicrobial activity of title compounds was examined against two gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), two gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes*) and three fungi (*Candida albicans*, *Aspergillus niger*, *Aspergillus clavatus*) using the broth microdilution method and antitubercular activity $H_{37}Rv$ using L. J. Slope Method. Several compounds exhibited good antibacterial activity (**26, 35, 39, 46** and **60** against *E. coli*; **36, 38** and **39** against *P. aeruginosa;* **17, 21, 41** and **50** against *S. aureus;* **28** *and* **37** *against S. pyogenes*); some displayed good antifungal activity (**22, 27, 29, 31, 36, 42, 43, 45, 46, 49, 50, 52, 64** and **65** against *C. albicans*). Compounds **42, 44, 46, 50, 58** and **63** showed good antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$.

INTRODUCTION:

Heterocyclic compounds, particularly five or six member ring compounds have taken the first place among various classes of organic compounds for their diverse biological activities. Nitrogen-containing heterocyclic plays an important character in medicinal chemistry and contributes to the society by helping in different life processes. Pyrimidine is a six-member heterocyclic compound that contains two nitrogen atoms at positions 1 and 3. The structure of the pyrimidine ring is similar to benzene and pyridine [1]. Pyrimidine does not exist in nature, but in the form of its various derivatives, it is found as a part of more complex system and is widely distributed. The presence of a pyrimidine base in thymine, cytosine, and uracil, which are the essential binding blocks of nucleic acids, DNA and RNA is one possible reason for their activity [2-4]. Pyrimidine derivatives are of interest because of their pharmacological properties. Literature indicates that compounds having pyrimidine nucleus have wide range of therapeutic uses that include anti-inflammatory[6], trimethoprim, sulfamethazine and sulphadiazine as antimicrobial[6], 5-fluorouracil as anticancer[7], idoxuridine and trifluoride as antiviral, zidovudine and stavudine as anti-HIV[8], sulfadoxine and pyrimethamine as antimalarial[9], minoxidil and prazosin as antihypertensive, sedatives and hypnotics[10], anticonvulsant, antihistaminic, cardiovascular[11] and toxoflavin and fervennuline as antibiotics. Biginelli in the year 1893 reported one-step synthesis of 3,4-dihydropyridine-2(1H)-one by three-component condensation of aldehydes, ethyl acetoacetate and urea in alcohol using strong mineral acid[12]. The method had certain limitations like low yield, longer reaction time, especially with aliphatic and substituted aromatic aldehydes etc. The scope of the original Biginelli reaction was gradually extended by variation of all three building blocks, allowing access to a large number of multi-functioned dihydropyrimidinones.

Another important pharmacophore moiety is 1,3,4-oxadiazole associated with a wide variety of therapeutically important drugs. 1,3,4-oxadiazole moiety has great attraction as bioisosteres for a number of biological targets due to their metabolic stability, ability to bind to target peptides and can be engaged in hydrogen bond formation. 1,3,4-oxadiazoles have been used in numerous therapeutic areas including anti-inflammatory[13], antimicrobial[14-15], anti-diabetes[16] and anti-cancer[17-18] there have been reports that 1,3,4-oxadiazoles substituted with other heterocyclic unit such as pyrazole[19] 1,2,3-triazole[20], and pyridine[21] also show potent biological activity. Along these lines, we became interested in

the synthesis of 1,3,4-oxadiazole derivatives equipped with a 1,2,3,4-tetrahydropyrimidine moiety as a heterocyclic unit.

Over a past decade, microwave-assisted chemistry has matured into a highly useful technique and provides an interesting alternative for heating chemical reactions. Microwave techniques in synthetic chemistry often elicit a dramatic increase of the reaction rate, is suited to increase demands of industry. It can generate the desired compound in a relatively short time, compared to conventional thermal heating conditions, and with low cost. A number of reviews and research articles [22-30] on the application of microwave technology in chemical synthesis have been published.

MATERIALS AND METHODS

All chemicals supplied by were Lobachem Ltd. and Ficher Scientific Ltd. Melting points were determined by the open tube capillary method and are uncorrected. TLC plates (silica gel G) checked the completion of the reaction and spots were visualized under UV radiation. IR spectra were recorded on Thermo Scientific Nicolet iS10 FT-IR spectrometer (KBr pellets) (γmax in cm^{-1}). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance II 400 NMR spectrometer (400 MHz) using TMS as internal standard DMSO-d_6 (δ in ppm). The mass spectra were recorded on micromass Q-T of micro (TOF MS ES+).The non-conventional reactions were conducted in a ‘‘QPro-M Modified Microwave Synthesis System’’ manufactured by the Questron Technologies Corporation, Ontario L4Z 2E9 Canada.

EXPERIMENTAL

Synthesis of ethyl 6-methyl-2-oxo-4-(4-propyl phenyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate. (16)

A mixture of 4-propyl benzaldehyde **(1)** (2.96 g, 20 mmol), ethyl acetoacetate **(14)** (5.2 g, 40 mmol), urea **(15)** (2.4 g, 40 mmol) and Conc. H_2SO_4 (1 mL) in absolute ethanol (10 mL) were taken in a borosilicate flask and kept inside the microwave oven for a period of 3-4 min (at 200 W). TLC using a mixture of toluene monitored the reaction progress: ethyl acetate (7:3) as the mobile phase. After the completion of the reaction, the reaction mixture was allowed to stand at room temperature and the product formed was filtered, washed with ethanol, water, dried and recrystallized from ethanol to obtain **(16).**

Other ethyl 6-methyl-2-oxo-4-(substituted)-1,2,3,4-tetrahydro pyrimidine-5-carboxylate **(17-28)** have been prepared from different aromatic aldehydes **(2-13)** by the same method.

16. Yield 76%; **IR (KBr, cm^{-1})**: 2932, 2835 (C-H, asym, sym), 1651 (C=O), 1604 (C=N), 1335 (C-N), 833 (C-S-C).

^{1}H NMR δ (ppm): 9.05 (s, 1H, -NH), 7.75 (s, 1H, -NH), 7.16-7.51 (m, 4H, aromatic), 4.61 (s, 1H, -CH (pyrimidine ring)), 3.97 (q, 2H, -CH_2 of ester), 2.94 (t, 2H, -CH_2 of propyl gorup), 2.27 (s, 3H, CH3), 1.70 (m, 2H, -CH_2 of propyl group), 1.10 (t, 3H, -CH_3 of ester), 0.86 (t, 3H, -CH_3 of propyl group).

Synthesis of 2-(6-methyl-2-oxo-4-(4-propyl phenyl)-1,2,3,4-tetrahydropyrimidine-5-carbonyl)hydrazine-1-carbothioamide. (29)

A mixture of compound **(16)** (6.04 g, 20 mmol) and thiosemicarbazide (2.73 g, 30 mmol) in DMF was exposed in MWI at 1 min intervals for 10-12 min (at 200-300 W). The progress of the reaction was periodically monitored by TLC using mobile phase toluene: methanol (9:1). After the completion of the reaction, the mixture was cooled to room temperature and poured into cold water. Thus the product obtained was filtered, washed with water, dried and recrystallized from ethanol to obtain compound **(29).**

Other 2-(6-methyl-2-oxo-4-(substituted)-1,2,3,4-tetrahydro pyrimidine-5-carbonyl)hydrazine-1-carbothioamide **(30-41)** have been prepared by the same method from 6-methyl-2-oxo-4-(substituted)-1,2,3,4-tetrahydro pyrimidine-5-carboxylate **(17-28)**.

29. Yield 73%; **IR (KBr, cm^{-1}):** 3337 (-NH_2), 3225, 3107 (-CONH), 2960, 2870 (-CH_3, asym, sym), 1686, 1631 (>C=O str), 1222 (-C=S str);

^{1}H NMR δ (ppm): 9.82 (s, 1H, -NHC=S), 9.27 (s, 1H, -NH_2), 9.04 (s, 1H, -NH), 8.98 (s, 1H, -NHC=O), 7.76 (s, 1H, -NH), 7.17-7.53 (m, 4H, aromatic), 4.68 (s, 1H, -CH (pyrimidine ring) 2.93 (t, 2H, -CH_2 of propyl gorup), 2.27 (s, 3H, CH_3), 1.69 (m, 2H, -CH_2 of propyl group), 0.86 (t, 3H, -CH_3 of propyl group).

Synthesis of 5-(5-amino-1,3,4-oxadiazol-2-yl)-6-methyl-4-(4-propyl phenyl)-3,4-dihydropyrimidine-2(1*H*)-one. (42)

Carbothioamide **(29)** (6.26 g, 20 mmol) in ethanol (5 mL) was added to 10% NaOH (20 mL) with cooling and shaking at 4 ^{0}C. To this clear solution, Iodine solution in KI (5%) was added gradually and shaking until the Iodine color persisted at room temperature. This reaction mixture was MWI for 12-30 min (at 300 W), the reaction progress was monitored by TLC using a mixture of toluene: ethyl acetate (7:3) as the mobile phase. The reaction mixture was concentrated and the residue was cooled and poured into ice cold water. This solution was filtered and acidified with 10% HCl to isolate the product. It was filtered, washed with water, dried and recrystallized from ethanol to obtain compound **(42).**

Other 5-(5-amino-1,3,4-oxadiazol-2-yl)-6-methyl-4-(substituted)-3,4-dihydro pyrimidin-2(1*H*)-one **(43-54)** have been prepared by the same method from 2-(6-methyl-2-oxo-4-(substituted)-1,2,3,4-tetrahydro pyrimidine-5-carbonyl)hydrazine-1-carbothioamide **(30-41)**.

42. Yield 76%; M.P. 112-114; **IR (KBr, cm^{-1}):** 3534 (-NH_2), 3240, 3114 (-CONH), 2935, 2856 (-CH_3, asym, sym), 1643 (>C=O str), 1274 (-C-O-C).

^{1}H NMR δ (ppm): 9.06 (s, 1H, -NH), 8.00 (s, 2H, -NH_2), 7.98 (s, 1H, -NH), 7.06-7.44 (m, 4H, aromatic), 5.15 (s, 1H, -CH (pyrimidine ring)), 2.93 (t, 2H, -CH_2 of propyl gorup), 2.25 (s, 3H, CH_3), 1.69 (m, 2H, -CH_2 of propyl group), 0.86 (t, 3H, -CH_3 of propyl group).

Anal. calcd. for **$C_{16}H_{19}N_5O_2$**: C, 61.33; H, 6.11; N, 22.35; Found: C, 61.30; H, 6.08; N, 22.31%.

Synthesis of (*E*)-2-((5-(6-methyl-2-oxo-4-(4-propyl phenyl)-1,2,3,4-tetrahydropyrimidin-5-yl)-1,3,4-oxadiazol-2-yl)diazenyl)malononitrile. (55)

5-Amino-1,3,4-oxadiazole derivative **(42)** (3.13 g, 10 mmol) was dissolved in a mixture of conc. H_2SO_4 (2.5 mL) and water (10 mL) and stirred until the temperature of the solution falls below 0 ^{0}C in an ice bath. To this solution, a cold aqueous solution of sodium nitrite (2.76 g, 40 mmol) was added gradually into the above cold solution with stirring till reaction mixture shows the positive test of nitrous acid on starch iodide paper and maintaining the temperature between 0 ^{0}C – 5 ^{0}C. Resulting diazonium salt solution was added to a stirred solution of malononitrile (0.66 g, 10 mmol) very slowly, maintaining the temperature

between 0 ^{0}C – 5 ^{0}C. The mixture was then stirred for 1hr at the same temperature. After completion of the reaction, the precipitates were filtered, washed with water and crystallized from absolute ethanol to get compound **(55)**.

Other ethyl (*E*)-2-((5-(6-methyl-2-oxo-4-(substituted)-1,2,3,4-tetrahydropyrimidin-5-yl)-1,3,4-oxadiazol-2-yl)diazenyl)malononitrile **(55-67)** have been prepared by the same method from 5-(5-amino-1,3,4-oxadiazol-2-yl)-6-methyl-4-(substituted)-3,4-dihydropyrimidin-2(1*H*)-one **(43-54).**

55. Yield 69%; M.P. 114-119; **IR (KBr, cm^{-1}):** 3224, 3157 (-CONH), 2961, 2871 (-CH_3, asym, sym), 2219 (-CN), 1422 (-N=N str), 1640 (>C=O str), 1222 (-C-O-C).

^{1}H NMR δ (ppm): 9.06 (s, 1H, -NH), 7.69 (s, 1H, -NH), 6.83-7.39 (m, 4H, aromatic), 5.18 (s, 1H, -CH (pyrimidine ring)), 3.98 (s, 1H, >CH-N=N-), 2.86 (t, 2H, -CH_2 of propyl gorup), 2.26 (s, 3H, -CH_3), 0.86 (t, 3H, -CH_3 of propyl group).

^{13}C NMR δ(ppm): 147.81 (C_{19}), 147.28 (C_{22}), 128.18 (C_{21}, C_{23}), 126.21 (C_{20}, C_{24}), 111.70 (C_1, C_3), 99.82 (C_{14}), 54.17 (C_{15}), 43.47 (C_2), 33.78 (C_{25}), 23.78 (C_{26}), 17.60 (C_{17}), 13.96 (C_{27}).

MS (EI) *m/z*: 390 (M^+).

Anal. calcd. for **$C_{19}H_{18}N_8O_2$:** C, 58.45; H, 4.65; N, 28.70; Found: C, 58.42; H, 4.62; N, 28.66%.

57. Yield 81%; M.P. 159-160**; IR (KBr, cm^{-1}):** 3221, 3156 (-CONH), 2960, 2870 (-CH_3, asym, sym), 2218 (-CN), 1421 (-N=N str), 1640 (>C=O str), 1221 (-C-O-C).

^{1}H NMR δ (ppm): 9.05 (s, 1H, -NH), 7.69 (s, 1H, -NH), 6.33-7.54 (m, 3H, aromatic), 5.17 (s, 1H, -CH (pyrimidine ring)), 3.98 (s, 1H, >CH-N=N-), 2.26 (s, 3H, CH_3).

^{13}C NMR δ(ppm): 157.22 (C_{19}), 149.32 (C_{22}), 117.76 (C_{21}), 113.61 (C_{20}), 111.73 (C_1, C_3), 99.82 (C_{14}), 54.14 (C_{15}), 43.46 (C_2), 17.60 (C_{17}).

MS (EI) *m/z*: 353 (M^+).

Anal. calcd. for **$C_{14}H_{10}N_8O_3$:** C, 49.71; H, 2.98; N, 33.12; Found: C, 49.68; H, 2.96; N, 33.09%.

59. Yield 76%; M.P. 201-203**; IR (KBr, cm^{-1}):** 3434 (-OH), 3222, 3157 (-CONH), 2961, 2871 (-CH_3, asym, sym), 2220 (-CN), 1423 (-N=N str), 1641 (>C=O str), 1222 (-C-O-C).

^{1}H NMR δ (ppm): 9.16 (s, 1H, -OH), 9.06 (s, 1H, -NH), 7.68 (s, 1H, -NH), 6.77-7.49 (m, 3H, aromatic), 5.17 (s, 1H, -CH (pyrimidine ring)), 3.99 (s, 1H, >CH-N=N-), 3.12 (s, 3H, -OCH_3), 2.27 (s, 3H, CH_3).

^{13}C NMR δ(ppm): 143.20 (C_{19}), 153.57 (C_{22}), 148.38 (C_{21}), 117.40 (C_{24}), 116.11 (C_{23}), 115.03 (C_1, C_3), 111.43(C_{20}), 99.39 (C_{14}), 43.47 (C_2), 55.42 (C_{15}), 55.36 (C_{26}), 17.64 (C_{17}).

MS (EI) *m/z*: 392 (M^+).

Anal. calcd. for **$C_{17}H_{14}N_8O_4$:** C, 51.78; H, 3.58; N, 28.42; Found: C, 51.75; H, 3.55; N, 28.40%.

60. Yield 73%; M.P. 172-173**; IR (KBr, cm^{-1}):** 3224, 3155 (-CONH), 2962, 2871 (-CH_3, asym, sym), 2219 (-CN), 1421 (-N=N str), 1639 (>C=O str), 1221 (-C-O-C).

^{1}H NMR δ (ppm): 9.05 (s, 1H, -NH), 7.67 (s, 1H, -NH), 6.81-7.54 (m, 4H, aromatic), 5.17 (s, 1H, -CH (pyrimidine ring)), 3.98 (s, 1H, >CH-N=N-), 2.24 (s, 3H, CH_3).

^{13}C NMR δ(ppm): 167.31 (C_{22}), 145.78 (C_{19}), 140.64 (C_{21}, C_{23}), 127.35 (C_{20}, C_{24}), 115.09 (C_1, C_3), 99.33 (C_{14}), 43.44 (C_2), 54.13 (C_{15}), 17.65 (C_{17}).

MS (EI) *m/z*: 366 (M^+).

Anal. calcd. for **$C_{16}H_{11}FN_8O_2$:** C, 52.46; H, 3.03; N, 30.59; Found: C, 52.43; H, 3.00; N, 30.56%.

64. Yield 92%; M.P. 174-175**; IR (KBr, cm^{-1}):** 3225, 3155 (-CONH), 2960, 2870 (-CH_3, asym, sym), 2218 (-CN), 1422 (-N=N str), 1638 (>C=O str), 1222 (-C-O-C).

^{1}H NMR δ (ppm): 9.08 (s, 1H, -NH), 7.67 (s, 1H, -NH), 6.84-7.52 (m, 4H, aromatic), 5.18 (s, 1H, -CH (pyrimidine ring)), 3.98 (s, 1H, >CH-N=N-), 2.26 (s, 3H, CH_3).

^{13}C NMR δ(ppm): 148.13 (C_{19}), 146.54 (C_{22}), 128.70 (C_{21}, C_{23}), 125.34 (C_{20}, C_{24}), 115.02 (C_1, C_3), 99.43 (C_{14}), 43.12 (C_2), 54.07 (C_{15}), 17.61 (C_{17}).

MS (EI) *m/z*: 382 (M^+), 384 (M^++2).

Anal. calcd. for **$C_{16}H_{11}ClN_8O_2$:** C, 50.21; H, 2.90; N, 29.28; Found: C, 50.17; H, 2.86; N, 29.26%.

65. Yield 94%; M.P. 192-193**; IR (KBr, cm^{-1}):** 3223, 3156 (-CONH), 2963, 2871 (-CH_3, asym, sym), 2219 (-CN), 1423 (-N=N str), 1641 (>C=O str), 1221 (-C-O-C).

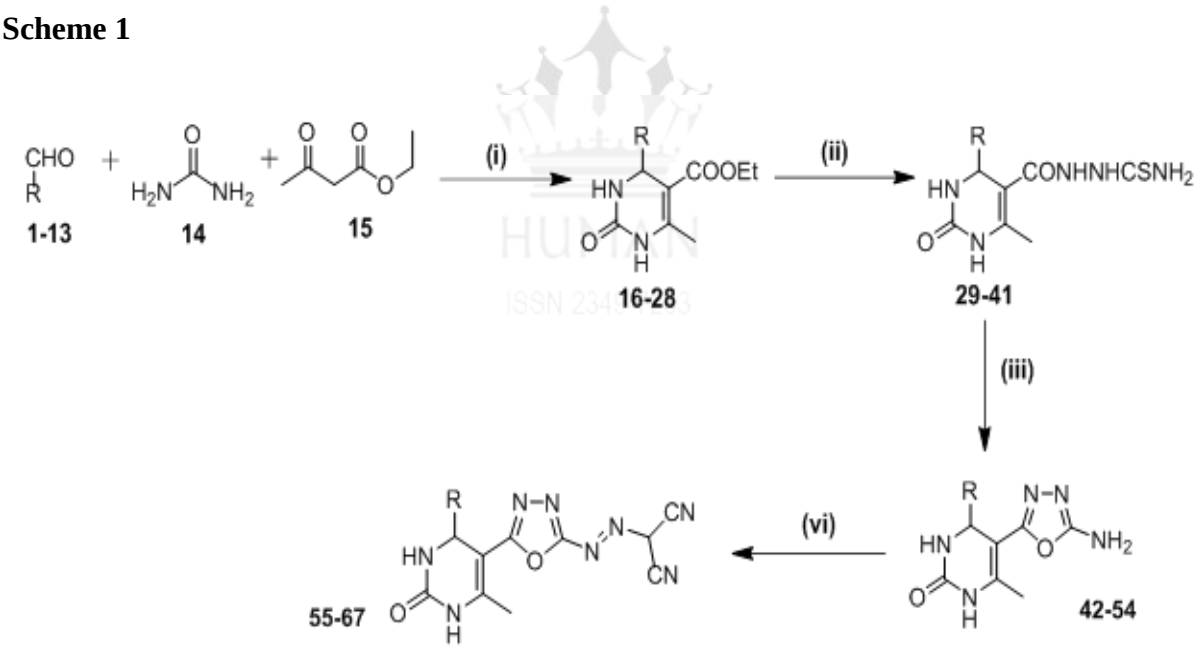
^{1}H NMR δ (ppm): 9.06 (s, 1H, -NH), 7.69 (s, 1H, -NH), 7.11-8.19 (m, 3H, aromatic), 5.18 (s, 1H, -CH (pyrimidine ring)), 3.99 (s, 1H, >CH-N=N-), 2.27 (s, 3H, CH_3).

^{13}C NMR δ(ppm): 146.63 (C_{19}), 138.18 (C_{21}), 132.14 (C_{22}), 126.31 (C_{20}), 115.07 (C_1, C_3), 99.41 (C_{14}), 43.15 (C_2), 56.75 (C_{15}), 17.64 (C_{17}).

MS (EI) *m/z*: 354 (M^+).

Anal. calcd. for **$C_{14}H_{10}N_8O_2S$:** C, 47.45; H, 2.84; N, 31.62; Found: C, 47.42; H, 2.81; N, 31.60%.

Scheme 1



R =	-C_6H_4-4-C_3H_7, -C_6H_4-3-Br, -C_4H_3O, -C_5H_4N, -C_6H_4-3-OH-4-OCH_3, -C_6H_4-4-F, -C_6H_4-4-OH, -C_6H_4-2-Cl, -C_6H_4-3-Cl, -C_6H_4-4-Cl, -C_4H_3S, -C_6H_4-4-$N(CH_3)_2$, -C_6H_5

(i) Conc. H_2SO_4, absolute ethanol, MWI 3-4 min (at 200 W); **(ii)** Thiosemicarbazide, DMF, MWI, 10-12 min (at 200-300 W); **(iii)** 10% NaOH, 4ºC, I_2 in KI (5%), MWI for 12-30 min (at 300 W); **(iv)** Nitrosyl sulfuric acid diazotization ($NaNO_2$ + H_2SO_4), coupling with ethyl acetoacetate, 0ºC – 5ºC; **(v)** Nitrosyl sulfuric acid diazotization ($NaNO_2$ + H_2SO_4), coupling with ethyl cynoacetate, 0ºC – 5ºC; **(vi)** Nitrosyl sulfuric acid diazotization ($NaNO_2$ + H_2SO_4), coupling with malononitrile, 0ºC - 5ºC.

RESULT, DISCUSSION AND CONCLUSION

Chemistry

ethyl 6-methyl-2-oxo-4-(substituted)-1,2,3,4-tetrahydropyrimidine-5-carboxylate**(16-28)** were synthesized by Biginelli reaction which is a three-component condensation of substituted aldehydes, ethyl acetoacetate and urea in alcohol using strong mineral acid as described in the literature. The synthetic route of the titled compounds**(55-67)** is outlined in Scheme 1 ethyl 6-methyl-2-oxo-4-(substituted)-1,2,3,4-tetrahydropyrimidine-5-carboxylate**(16-28)** on reaction with thiosemicarbazide in DMF, which was followed by the cyclization reaction using 10% NaOH, and I_2 in KI (5%) at 4^0C to give 5-(5-amino-1,3,4-oxadiazol-2-yl)-6-methyl-4-(substituted)-3,4-dihydropyrimidin-2(1*H*)-one **(42-54)** via microwave irradiation**,** further synthesized compounds**(42-54)** were under go diazotization ($NaNO_2$ + H_2SO_4), which were coupled with malononitrile to yield (*E*)-2-((5-(6-methyl-2-oxo-4-(substituted)-1,2,3,4-tetrahydropyrimidin-5-yl)-1,3,4-oxadiazol-2-yl)diazenyl)malononitrile**(55-67).**

IR spectra of **42** showed the broad stretching band around 3534cm^{-1} for NH_2, which disappeared in the IR spectra of **55**. In the same way, ^{1}H NMR spectrum of **42** showed a singlet at δ 8.00, which were accounted for NH_2, which disappeared in ^{1}H NMR spectrum of **55,** that confirming the formation of the final product.

Biology

In vitro Antimicrobial Activity

All the compounds **16-67** were assayed for their antimicrobial activity against two Gram-negative bacteria (*E. coli*, *P. aeruginosa*), two Gram-positive bacteria (*S. aureus*, *S. pyogenes*) and three fungi (*C. albicans*, *A. niger*, *A. clavatus*) using the broth microdilution

method and the Minimum Inhibitory Concentration value are reported in **Table 1-4**. Ampicillin, Ciprofloxacine, Chloramphenicol, Greseofulvin, Nystatin were used as standard drug.

The investigation of antimicrobial screening data reveals several compounds are active and showing moderate to good antimicrobial activity. Amongst them, Compound significantly more potent were **26**, **35**, **39** and **46** at 62.5 µg/mL; **21**, **23**, **25**, **27** and **28** at 100-125 µg/mL against *E. coli*. Further, Compounds **36** and **38** exhibited significant activity at 50 µg/mL; **23, 28, 39, 41, 46** and **51** exhibited good activity at 62.5-100 µg/mL against *P. aeruginosa.* Compounds **17, 18, 21, 22, 30, 37, 41, 50, 51** and **64** were shown good activity 62.5-100 µg/mL against *S. aureus*. Compounds showed promising activity were **28, 37** at 62.5 µg/mL; **17, 36, 42, 46, 63** and **64** exhibited good activity at 100 µg/mL against *S. pyogenes.* Compounds found to be potent were **29** and **50** at 100 µg/mL against *C. albicans,* when compared with Nystatin and Greseofulvin. Compounds **22, 27, 31, 36, 42, 43, 45, 46, 49, 52, 64** and **65** at 250 µg/mL; **44, 47, 51, 58, 59, 60** and **62** at 500 µg/mL exhibited good activity against *C. albicans*, when compared with only Greseofulvin .

***In vitro* Anti-Tubercular Activity**

The encouraging results from the antibacterial studies impelled us to go for preliminary screening of synthesized compounds against *M. tuberculosis* are summarized in Table 5. From the preliminary examination of the antitubercular activity results, Compounds found to be good active were **42**, **44, 46, 50** and **58** at 62.5 µg/mL and derivatives **45**, **51** demonstrated moderate activity at 100 µg/mL against *M. tuberculosis* $H_{37}R_v$ when compared with Rifampicin.

Table 1: Antibacterial and antifungal activity of compounds 16-28

Compound No.	Minimum Inhibitory Concentration (µg/ml)						
	Gram-negative bacteria		Gram-positive bacteria		Fungal species		
	E. coli MTCC-443	*P. aeruginosa* MTCC-741	*S. aureus* MTCC-96	*S. pyogenes* MTCC-442	*C. albicans* MTCC-227	*A. niger* MTCC-282	*A. clavatus* MTCC-1323
16	250	250	250	200	1000	200	1000
17	250	250	62.5	100	1000	250	>1000
18	200	250	100	250	1000	1000	>1000
19	200	500	250	250	1000	>1000	500
20	250	250	500	200	500	>1000	500
21	100	250	62.5	125	500	500	250
22	200	250	100	500	250	1000	1000
23	100	100	200	250	1000	1000	500
24	200	250	200	1000	>1000	1000	1000
25	100	200	250	>1000	>1000	200	1000
26	62.5	500	250	>1000	500	1000	500
27	100	250	100	500	250	500	1000
28	125	100	200	250	1000	1000	250
Ampiciline	100	100	250	100	-	-	-
Ciproflox-acine	25	25	50	50	-	-	-
Chloramp-henicol	50	50	50	50	-	-	-
Greseseof-ulvin	-	-	-	-	500	100	100
Nystatin	-	-	-	-	100	100	100

Table 2: Antibacterial and antifungal activity of compounds 29-41

Compound No.	Minimum Inhibitory Concentration (µg/ml)						
	Gram-negative bacteria		Gram-positive bacteria		Fungal species		
	E. coli MTCC-443	*P. aeruginosa* MTCC-741	*S. aureus* MTCC-96	*S. pyogenes* MTCC-442	*C. albicans* MTCC-227	*A. niger* MTCC-282	*A. clavatus* MTCC-1323
29	200	500	1000	200	100	>1000	1000
30	250	200	100	500	500	>1000	250
31	250	250	500	125	250	500	1000
32	200	250	500	200	1000	1000	500
33	125	125	200	250	>1000	1000	1000
34	500	500	125	500	>1000	1000	1000
35	62.5	500	500	250	500	200	>1000
36	500	50	1000	100	250	1000	>1000
37	250	250	100	62.5	1000	1000	>1000
38	500	50	250	250	1000	1000	500
39	62.5	62.5	250	200	500	500	1000
40	125	500	125	200	1000	500	1000
41	500	100	62.5	200	>1000	200	500
Ampiiline	100	100	250	100	-	-	-
Ciproflox-acine	25	25	50	50	-	-	-
Chloramp-henicol	50	50	50	50	-	-	-
Greseseof-ulvin	-	-	-	-	500	100	100
Nystatin	-	-	-	-	100	100	100

Table 3: Antibacterial and antifungal activity of compounds 42-54

Compound No.	Minimum Inhibitory Concentration (µg/ml)						
	Gram-negative bacteria		Gram-positive bacteria		Fungal species		
	E. coli MTCC-443	*P. aeruginosa* MTCC-741	*S. aureus* MTCC-96	*S. pyogenes* MTCC-442	*C. albicans* MTCC-227	*A. niger* MTCC-282	*A. clavatus* MTCC-1323
42	200	200	250	100	250	500	1000
43	100	200	200	250	250	>1000	>1000
44	200	250	500	250	500	200	500
45	100	250	250	200	250	200	1000
46	62.5	100	125	100	250	250	1000
47	100	250	125	200	500	1000	1000
48	125	200	200	125	1000	>1000	200
49	200	500	250	500	250	>1000	1000
50	500	250	62.5	250	100	500	500
51	500	100	100	200	500	1000	1000
52	250	125	250	500	250	1000	1000
53	200	200	500	125	1000	1000	500
54	250	250	500	200	>1000	200	1000
Ampiiline	100	100	250	100	-	-	-
Ciproflox-acine	25	25	50	50	-	-	-
Chloramp-henicol	50	50	50	50	-	-	-
Greseseof-ulvin	-	-	-	-	500	100	100
Nystatin	-	-	-	-	100	100	100

Table 4: Antibacterial and antifungal activity of compounds 55-67

Compound No.	Minimum Inhibitory Concentration (µg/ml)						
	Gram-negative bacteria		Gram-positive bacteria		Fungal species		
	E. coli MTCC-443	*P. aeruginosa* MTCC-741	*S. aureus* MTCC-96	*S. pyogenes* MTCC-442	*C. albicans* MTCC-227	*A. niger* MTCC-282	*A. clavatus* MTCC-1323
55	250	500	250	500	1000	>1000	>1000
56	500	500	250	250	>1000	>1000	>1000
57	250	250	250	250	>1000	500	500
58	500	500	1000	200	500	1000	1000
59	500	250	250	250	500	1000	1000
60	100	250	250	250	500	500	500
61	500	500	250	500	1000	500	1000
62	250	200	500	200	500	1000	1000
63	200	200	125	100	1000	500	500
64	125	200	100	100	250	500	1000
65	500	500	125	500	250	1000	500
66	125	125	500	500	1000	500	500
67	200	200	250	200	1000	500	250
Ampiline	100	100	250	100	-	-	-
Ciproflox-acine	25	25	50	50	-	-	-
Chloramp-henicol	50	50	50	50	-	-	-
Greseseof-ulvin	-	-	-	-	500	100	100
Nystatin	-	-	-	-	100	100	100

Table 5: Antitubercular activity of compounds 42-67

Compound	MIC values (μg/mL) of *M. tuberculosis H37Rv*	% Inhibition
42	62.5	99
43	500	98
44	62.5	97
45	100	99
46	62.5	99
47	250	98
48	500	99
49	1000	98
50	62.5	98
51	100	99
52	500	99
53	1000	98
54	500	98
55	250	98
56	500	98
57	1000	98
58	62.5	99
59	100	98
60	500	98
61	100	99
62	250	99
63	62.5	99
64	100	97
65	100	99
66	500	98
67	1000	98
Isoniazide	0.2	99
Rifampicin	40	99

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