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Research Article

SYNTHESIS AND ANTI-MICROBIAL ACTIVITY OF SOME SUBSTITUTED 1-(1-(6-METHYL-2-OXO-4-PHENYL-1,2,3,4-TETRAHYDROPYRIMIDIN-5-YL)ETHYLIDENE)THIOSEMICARBAZIDE

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ABSTRACT

The pharmacological properties of thiosemicarbazide and pyrimidine derivatives, we have in the present study, synthesis of substituted 1-(1-(6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidin-5-yl)ethylidene)thiosemicarbazide are carried out by refluxing 5-acetyl-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one and thiosemicarbazide in equimolar ratio in the presence of alcohol, acetic acid. The chemical structures of the synthesized compounds were confirmed by means of IR, ^{1}H-NMR, Mass spectral and Elemental analysis. These compounds were screened for anti-bacterial (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginonasa* ATCC 27853) and anti-fungal (*Candida albicans*) activities by cup-plate technique. Antimicrobial activities of the compounds were also determined at different levels of concentration. Most of the synthesized compounds exhibited mild to moderate anti-bacterial and anti-fungal activities.

Keywords: Pyrimidines, Thiosemicarbazide, Anti-Bacterial and Anti-Fungal activities.

INTRODUCTION

Recently compounds with two heteroatoms in the ring have been intensively investigated. Heterocyclic thiones and thiosemicarbazide, which contain chemically active N(H)C(S) or =NN(H)C(S) group, are useful model compounds for sulfur-containing analogues of purine and pyrimidine bases. In view of the pharmacological properties of thiosemicarbazide, pyrimidine derivatives and heterocyclic annulated pyrimidines continue to attract great interest due to the wide variety of interesting biological activities observed for these compounds, such as anticancer [1], antiviral [2], antitumor [3], anti-inflammatory [4], antimicrobial [5], antifungal [6], antihistaminic [7] and analgesic [8] activities. Thiosemicarbazide and their derivatives form an important class of organic compounds due to their structural chemistry and biological activities, such as antibacterial, antivirus activities and cerebral infarction (Free radical scavenger) [9]. Thiosemicarbazide derivatives are reported to show biological activity, including antifungal, anti-HIV, analgesic, anti-inflammatory and anti-tumor effects [10-16]. It is also reported for dielectric studies [17]. Looking to the usefulness and importance of thiosemicarbazide and pyrimidine, it was considered worthwhile to the synthesis hybrid scaffolds.

MATERIAL AND METHODS

All solvents were distilled prior to use. TLC was performed on silica gel G (Qualigen). Melting points were determined by open capillary method and are uncorrected. ^{1}H NMR spectra were recorded in $CDCl_3$/DMSO-d_6 solution on a Brucker Avance II 400 NMR Spectrometer. Chemical shifts are reported in ppm using TMS as an internal standard. IR spectra were obtained on a Shimadzu FT-IR spectrophotometer using KBr discs. Mass spectra were recorded by using Shimadzu gas chromatograph.

Synthesis of substituted 1-(1-(6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidin-5-yl)ethylidene)thiosemicarbazide (2a-i):

A mixture of substituted 5-acetyl-3,4-dihydro-6-methyl-4-phenylpyrimidin-2(1H)-one (0.01 mol), thiosemicarbazide (0.01 mol), 30 mL of ethanol and 5mL acetic acid was added. The mixture was refluxed on water bath at 80-90^{0}C for 5-6 hr. The progress of reaction was monitor by TLC. The excess of ethanol was distilled off and reaction mixture was pour in ice-cold water to isolate solid product. Further crystallized from methanol-acetic acid. Physical characterization data depicted in (Table 1).

Table 1: Physical characterization data of synthesized new compound (2a-i)

Entry	R_1	R_2	X	Molecular formula	MW	M.P. (^{0}C)	Yield[A] (%)
2a	H	H	O	$C_{14}H_{17}N_5OS$	303	180	87
2b	OCH_3	H	O	$C_{15}H_{19}N_5O_2S$	333	124	79
2c	OH	H	O	$C_{14}H_{17}N_5O_2S$	319	119	75
2d	Cl	H	O	$C_{14}H_{16}ClN_5OS$	337	125	91
2e	H	Cl	O	$C_{14}H_{16}ClN_5OS$	337	182	90
2f	H	H	S	$C_{14}H_{17}N_5S_2$	319	201	89
2g	OCH_3	H	S	$C_{15}H_{19}N_5OS_2$	349	146	76
2h	OH	H	S	$C_{14}H_{17}N_5OS_2$	335	209	78
2i	H	Cl	S	$C_{14}H_{16}ClN_5S_2$	353	213	85

[A] Isolated Yield

Anti-microbial activity

All the title compounds were screened for their anti-bacterial and anti-fungal activities. The antibacterial activity of the synthesized compounds were tested against one gram positive bacteria (*Staphylococcus aureus* ATCC 25923) and two gram negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginonasa* ATCC 27853) using Muller-Hinton agar medium (Hi-Media Laboratories, India). The anti-fungal activities of the compounds were tested against one fungi namely *Candida albicans* using Muller-Hinton agar medium (Hi-Media Laboratories, India). For preliminary screening, the anti-microbial tests were carried out by the cup-plate method. Antimicrobial activities of the compounds were also determined at different levels of concentration.

Antibacterial study

The antibacterial activity of compounds **(2a-i)** were assayed at different level of concentration (25, 50, 100 µg/mL) in solvent DMSO against strains of gram +ve and gram –ve pathogenic bacteria (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginonasa)*. Initially, susceptibility testing were carried out by measuring the inhibitory zone diameter on Muller-Hinton agar, with conventional cup-plate method. The plates were incubated at 37.5^{0}C for 24 hr and the inhibitory zone diameters were measured in millimeter (mm). The inhibitory effects of compounds **(2a-i)** against these organisms are depicted in Table 2. The results were compared with Doxicycline and Ampicillin.

Antifungal study

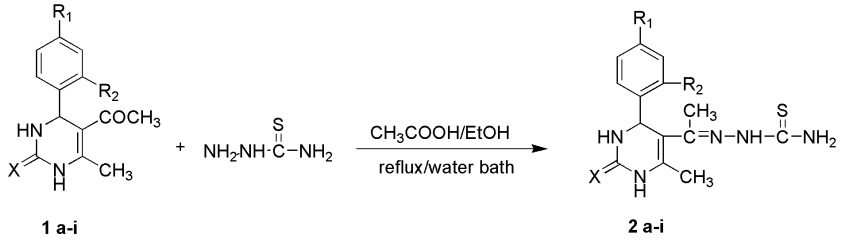
The antifungal activities of compounds **(2a-i)** were assayed in vitro at different level of concentration (25, 50, 100 µg/mL) in solvent DMSO against *C. albicans*. Fluconazole was used as standard fungicide for the antifungal test. Muller-Hinton agar was used as basal medium for test fungi, Screening was carried out by conventional cup-plate method. The plates were then incubated at 37.5^{0}C for 48 hours. The zone of inhibition were measured in mm. (Table 2)

Table 2: Antimicrobial–screening results of synthesized new compound (2a-i)

Entry	Bacterial Strain									Fungal Strain		
	E. Coli			*P. Aeruginonasa*			*S. Aureus*			*C. Albican*		
	100 µg	**50 µg**	**25 µg**	**100 µg**	**50 µg**	**25 µg**	**100 µg**	**50 µg**	**25 µg**	**100 µg**	**50 µg**	**25 µg**
2a	13	11	9	10	8	-	13	10	-	14	11	9
2b	12	10	8	9	7	-	13	10	-	13	10	8
2c	12	10	8	9	7	-	12	10	-	13	10	8
2d	11	10	8	8	6	-	10	9	-	12	10	8
2e	13	11	9	10	8	-	11	9	-	14	11	9
2f	11	9	8	9	7	-	12	9	-	12	10	8
2g	12	10	8	9	7	-	12	10	-	13	11	8
2h	13	10	8	10	8	-	13	10	-	12	10	8
2i	13	11	9	10	8	-	12	9	-	14	11	9
Ampiciline	18	15	12	15	12	9	32	29	24	-	-	-
Doxicycline	35	30	20	14	12	10	36	32	25	-	-	-
Fluconazole	-	-	-	-	-	-	-	-	-	33	30	11

RESULT AND DISCUSSION

In this paper, we would like to report the reactivity of substituted 5-acetyl-3,4-dihydro-6-methyl-4-phenylpyrimidin-2(1H)-one(**1a-i**) with thiosemicarbazide. The reaction of compounds **1a-i** with thiosemicarbazide in the presence of ethanol, acetic acid afforded the respective substituted 1-(1-(6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidin-5-yl)ethylidene)thiosemicarbazide (**2a-i**) as only separated product in high yields in a one-step procedure (Scheme 1).



Scheme 1

Chemistry

1-(1-(6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidin-5-yl)ethylidene)thiosemicarbazide 2a.

m.p.: 180 ^{0}C. IR (KBr): ν_{max}cm^{-1} 3350-3450 (NH_2 & NH), 3017 (Ar-CH), 2915 (CH in CH_3), 1720 (C=O), 1518 (C=N), 1462 (C=C), 653 (C-S). ^{1}H-NMR (DMSO-d_6) : δ 1.03 (s, 3H, CH_3), 2.30 (s, 3H,Ar-CH_3), 4.16 (s, 2H, NH_2), 5.56 (s, 1H, CH), 7.05-7.10 (m, 3H, Ar-CH), 7.12-7.26 (m, 2H, Ar-CH), 7.00 (s, 1H, NH). MS (m/z): 303M$^+$. Elemental analysis: Calculated for ($C_{14}H_{17}N_5OS$) C: 55.42; H: 5.65; N: 23.08. found C:52.56; H:5.69; N:23.24.

1-(1-(4-(4-methoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidin-5-yl)ethylidene)thiosemicarbazide 2b.

m.p.: 124 ^{0}C. IR (KBr): ν_{max}cm^{-1} 3345-3460 (NH_2 & NH), 3041 (Ar-CH), 2920 (CH in CH_3), 1725 (C=O), 1514 (C=N), 1457 (C=C). ^{1}H-NMR : δ 1.13 (s, 3H, CH_3), 2.2 (s, 3H, Ar-CH_3), 3.73 (s, 3H, OCH_3) 4.18 (s, 2H, NH_2), 5.60 (s, 1H, CH), 7.00-7.12 (dd, 2H, Ar-CH), 7.24-7.30 (dd, 2H, Ar-CH), 7.00 (s, 1H, NH). MS (m/z): 333M$^+$. Elemental analysis: Calculated for ($C_{15}H_{19}N_5O_2S$) C: 54.04; H: 5.74; N: 21.01. found C:54.20; H:5.75; N:21.10.

1-(1-(4-(4-hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidin-5-yl)ethylidene)thiosemicarbazide 2c

m.p.: 119 ^{0}C. IR (KBr): ν_{max}cm^{-1} 3500 (OH), 3367-3489 (NH_2 & NH), 3032 (Ar-CH), 2926 (CH in CH_3), 1764 (C=O), 1522 (C=N), 1453 (C=C). ^{1}H-NMR : δ 1.12 (s, 3H, CH_3), 2.01 (s, 3H, Ar-CH_3), 4.30 (s, 2H, NH_2), 5.60 (s, 1H, CH), 6.61-6.72 (dd, 2H, Ar-CH), 6.75-6.82 (dd, 2H, Ar-CH), 7.01 (s, 1H, NH), 9.86 (s, 1H, OH). MS (m/z): 319M$^+$. Elemental analysis: Calculated for ($C_{14}H_{17}N_5O_2S$) C: 52.65; H: 5.37; N: 21.93; found C:52.42; H:5.39; N:22.02.

1-(1-(4-(4-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidin-5-yl)ethylidene)thiosemicarbazide 2d.

m.p.: 125 ^{0}C. IR (KBr): ν_{max}cm^{-1} 3389-3450 (NH_2 & NH), 3040 (Ar-CH), 2935 (CH in CH_3), 1722 (C=O), 1519 (C=N), 1448 (C=C). ^{1}H-NMR : δ 1.12 (s, 3H, CH_3), 2.2 (s, 3H, Ar-CH_3), 4.18 (s, 2H, NH_2), 5.78 (s, 1H, CH), 7.01 (s, 1H, NH), 7.02-7.10 (dd, 2H, Ar-CH), 7.31-7.45 (dd, 2H, Ar-CH). MS (m/z): 337M$^+$. Elemental analysis: Calculated for ($C_{14}H_{16}ClN_5OS$) C: 49.77; H: 4.76; N: 20.72; found C:49.56; H:4.89; N:20.87.

1-(1-(4-(2-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidin-5-yl)ethylidene)thiosemicarbazide 2e.

m.p.: 182 ^{0}C. IR (KBr): ν_{max}cm^{-1} 3320-3428 (NH_2 & NH), 3018 (Ar-CH), 2912 (CH in CH_3), 1724 (C=O), 1532 (C=N), 1463 (C=C). ^{1}H-NMR : δ 1.3 (s, 3H, CH_3), 2.2 (s, 3H, Ar-CH_3), 4.02 (s, 2H, NH_2), 5.84 (s, 1H, CH), 7.01 (s, 1H, NH), 7.12-7.30 (m, 3H, Ar-CH), 7.20-7.28 (m, 1H, Ar-CH). MS (m/z): 337M$^+$. Elemental analysis: Calculated for ($C_{14}H_{16}ClN_5OS$) C: 49.77; H: 4.76; N: 20.72. found C:49.68; H:4.80; N:20.80

1-(1-(6-methyl-4-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)ethylidene)thiosemicarbazide 2f.

m.p.: 201 ^{0}C. IR (KBr): ν_{max}cm^{-1} 3364-3412 (NH_2 & NH), 3048 (Ar-CH), 2932 (CH in CH_3), 1726 (C=O), 1513 (C=N), 1446 (C=C). ^{1}H-NMR : δ 1.23 (s, 3H, CH_3), 2.29 (s, 3H, Ar-CH_3), 4.23 (s, 2H, NH_2), 5.19 (s, 1H, CH), 6.89 (s, 1H, NH), 7.33-7.45 (m, 3H, Ar-CH), 7.24-7.31 (m, 2H, Ar-CH). MS (m/z): 319M$^+$. Elemental analysis: Calculated for ($C_{14}H_{17}N_5S_2$) C: 52.64; H: 5.35; N: 21.93; found C:52.50; H:5.40; N:22.06.

1-(1-(4-(4-methoxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)ethylidene)thiosemicarbazide 2g.

m.p.: 146 ^{0}C. IR (KBr): ν_{max}cm^{-1} 3345-3489 (NH_2 & NH), 3036 (Ar-CH), 2926 (CH in CH_3), 1722 (C=O), 1520 (C=N), 1460 (C=C). ^{1}H-NMR : δ 0.95 (s, 3H, CH_3), 2.1 (s, 3H, Ar-CH_3), 3.86 (s, 3H, OCH_3), 4.31 (s, 2H, NH_2), 5.65 (s, 1H, CH), 6.98 (s, 1H, NH), 7.02-7.12 (dd, 2H, Ar-CH), 7.13-7.26 (dd, 2H, Ar-CH). MS (m/z): 349M$^+$. Elemental analysis: Calculated for ($C_{15}H_{19}N_5OS_2$): C: 51.54; H: 5.47; N: 20.06; found C:51.40; H:5.50; N:20.14.

1-(1-(4-(4-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)ethylidene)thiosemicarbazide 2h.

m.p.: 209 ^{0}C. IR (KBr): ν_{max}cm^{-1} 3512 (OH), 3386-3488 (NH_2 & NH), 3024 (Ar-CH), 2939 (CH in CH_3), 1720 (C=O), 1526 (C=N), 1452 (C=C). ^{1}H-NMR : δ1.25 (s, 3H, CH_3), 1.68 (s, 3H, CH_3), 4.20 (s, 2H, NH_2), 5.88 (s, 1H, CH), 6.60-6.72 (dd, 2H, Ar-CH), 6.82-6.96 (dd, 2H, Ar-CH), 7.00 (s, 1H, NH), 10.01 (s, 1H, OH). MS (m/z): 335M$^+$. Elemental analysis: Calculated for ($C_{14}H_{17}N_5OS_2$) C: 50.12; H: 5.12; N: 20.89; found C:50.30; H:5.24; N:20.94.

1-(1-(4-(2-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)ethylidene)thiosemicarbazide 2i.

m.p.: 213 ^{0}C. IR (KBr): ν_{max}cm^{-1} 3374-3450 (NH_2 & NH), 3019 (Ar-CH), 2917 (CH in CH_3), 1726 (C=O), 1518 (C=N), 1454 (C=C). ^{1}H-NMR : δ1.04 (s, 3H, CH_3), 2.2 (s, 3H, Ar-CH_3), 4.44 (s, 2H, NH_2), 5.78 (s, 1H, CH), 7.13-7.23 (m, 3H, Ar-CH), 7.45-7.60 (m, 1H, Ar-CH), 7.70 (s, 1H, NH). MS (m/z): 353M$^+$. Elemental analysis: Calculated for ($C_{14}H_{16}ClN_5S_2$) C: 47.53; H: 4.56; N: 19.78; found C:47.60; H:4.70; N:19.80.

Anti-microbial activity

Most of the synthesized compound exhibited mild to moderate antibacterial and anti-fungal activity against the tested microorganism when compared to standard drug (Doxicycline, Ampicillin and Fluconazole for anti-bacterial and anti-fungal respectively). The entire synthesized compound exhibited mild to moderate anti-microbial activity at different levels of concentration.

CONCLUSIONS

An efficient synthesis of substituted 1-(1-(6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidin-5-yl)ethylidene)thiosemicarbazide 2a-i. The biological evaluation of activities of substituted 1-(1-(6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidin-5-yl)ethylidene)thiosemicarbazide 2a-i exhibited mild to moderate anti-microbial activity at different levels of concentration against *Escherichia Coli, Pseudomonas aeruginonasa, Staphylococcus aureus and Candida albican*. Work is underway in our laboratories to increase the efficacy and specificity of the titled scaffolds as xenobiotics by structural refinements and modulation.

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