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Original research paper

Simple approach to thieno[3,2-*d*]pyrimidines as new scaffolds of antimicrobial activities

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6′-(4-Chlorophenyl)-spiro[cyclohexane-1,2′-thieno[3,2-*d*][1,3]oxazin]-4′(1′*H*)-one (**1**) was synthesized and used as a starting material for the synthesis of a novel series of spiro compounds having biologically active sulfonamide **2a-e** and 3′-(4-acetylphenyl)-6′-(4-chlorophenyl)-1′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidine-4′(3′*H*)-one (**3**). Compound **2a** was used as a key intermediate for the synthesis of sulfonyl carbothioamide derivatives **4a-c**. Also, compound **3** was used as an intermediate for the synthesis of 3′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-3′-yl]phenyl}-2-imino-4-(substituted phenyl and/or thienyl)-1,2-dihydropyridine-3-carbonitrile derivatives **5a-e**, 3′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-3′-yl]phenyl}-2-oxo-4-(substituted phenyl and/or thienyl)-1,2-dihydropyridine-3-carbonitrile derivatives **6a-e**, and 4-[(2*Z*)-3-substituted-arylprop-2-enoyl]phenyl-1′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidine derivatives **7a-e**. Cyclocondensation of **7a-e** with hydrazine hydrate produced 6′-(4-chlorophenyl)-3′-[4-(5-substituted aryl-4,5-dihydro-1*H*-pyrazol-3-yl)phenyl]-1′*H*-spiro[cyclohexane-1,2′-thieno-[3,2-*d*]pyrimidin]-4′(3′*H*)-ones **8a-e** but with hydroxylamine hydrochloride afforded the corresponding isoxazoline derivatives **9a-e**. Also, cyclocondensation by thiourea afforded 2-thioxo-1,2-dihydropyrimidin-4-yl)-phenyl-spiro-{cyclohexanethieno[3,2-*d*]pyrimidin}-4-one derivatives **10a-e**. The new compounds were investigated for antimicrobial activity. Compounds **2c**, **8b**,**c**, **9b** and **10b** were the most potent ones against both Gram-negative and Gram-positive bacteria. Compound **8c** exhibited higher antifungal activity towards the examined fungi with *MIC* of 1–2 μmol mL^{-1} compared to ketoconazole (*MIC* 2–3 μmol mL^{-1}).

Keywords: thieno[3,2-*d*][1,3]oxazin]-4′-one, thieno[3,2-*d*]pyrimidines, *N*-nucleophiles, spiro-thieno[3,2-*d*]pyrimidine, ring transformation, antimicrobial

Thienopyrimidines make an important class of heterocyclic compounds, which have attracted a great deal of attention due to their wide range of chemotherapeutic activities, including antimicrobial (1–4), moderate activity against tumor cell proliferation *in vitro*

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(5–7), antiviral (8, 9), analgesic and anti-inflammatory (10–13), antihistaminic (14) and anticonvulsant activities (15). With a variety of derivatives obtained by annulations and functional group manipulations, many thieno[3,2-*d*]-pyrimidine derivatives occupy a special place because of their biological activities such as kinase (16–18) and phosphodiesterase IV inhibiting (19, 20), gastric antisecretory (21) and antifolating (22) ones. Furthermore, several pyridone and imino pyridine derivatives are of chemotherapeutic importance (23).

The combinations of thieno[3,2-*d*]pyrimidines with a variety of sulfa drugs and heterocycles might add a new biological, antibacterial and antifungal action. To the best of our knowledge, antibacterial and antifungal activity of thieno[3,2-*d*]pyrimidines has not been reported before.

EXPERIMENTAL

All melting points were taken on an Electrothermal IA 9100 series digital melting point apparatus (Shimadzu, Japan). Microanalytical data were obtained with the aid of a Vario Elementar Apparatus (Shimadzu). Elemental analyses of all compounds were within ± 0.4 % of the theoretical values (Table I). The IR spectra (KBr) were recorded on a Perkin-Elmer 1650 spectrometer (Perkin-Elmer, USA). The ^{1}H and ^{13}C NMR spectra were measured with a Jeol ECA 500 MHz instrument (Jeol, Japan) in DMSO-d_6 and chemical shifts were recorded in δ ppm relative to TMS. Mass spectra (EI) were run at 70 eV with a Finnigan SSQ 7000 spectrometer (Thermo-Instrument System Incorporation, USA). Purity of the compounds was checked on aluminium plates coated with silica gel (Merck, Germany).

3-Amino-5-(4-chlorophenyl)-thiophene-2-carboxylic acid, *p*-amino-acetophenone, ciprofloxacin and ketoconazole were obtained from Sigma-Aldrich (USA). Chemicals and solvents (Analar ≥ 99 %) were purchased from Sigma-Aldrich.

Syntheses

*6′-(4-Chlorophenyl)-spiro[cyclohexane-1,2′-thieno[3,2-*d*][1,3]oxazin]-4′(1′*H*)-one (**1**).* – The compound was obtained from 3-amino-5-(4-chlorophenyl)thiophene-2-carboxylic acid (2.54 g, 0.01 mol) and cyclohexanone (1.24 g, 0.01 mol) refluxed in basic medium (ethanol and piperidine). The solid that separated upon cooling was filtered off in the form of white crystals.

*Spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-3′-yl]benzenesulfonamides (**2a-e**). General procedure.* – A mixture of **1** (3.33 g, 0.01 mol) and 0.01 mol of the appropriate sulfa drug (sulfanilamide, sulfaguanidine, sulfadiazine, sulfapyridine, sulfamethoxazole) in 20 mL glacial acetic acid containing anhydrous sodium acetate (1.64 g, 0.02 mol) was refluxed for 20 h. Upon pouring on crushed ice/water, crystals were obtained, filtered, washed with water and recrystallized from a proper solvent to give **2a-e**, respectively. The following compounds were prepared: 4-[6′-(4-chlorophenyl)-4′-oxo-1′,4′-dihydro-3′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin-3′-yl]-benzenesulfonamide (**2a**), 3′-(4-{[amino(imino)methyl] sulfonyl}phenyl)-6′(4-chlorophenyl)-1′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-4′(3′*H*)-one (**2b**), 4-[6′-(4-chloro-phenyl)-4′-oxo-1′,4′-dihydro-3′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-3′-yl]-*N*-pyrimidin-2-yl]-benzenesulfonamide (**2c**), 4-[6′-(4-chlorophenyl)-4′-oxo-1′,4′-dihydro-3′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-3′-yl]-*N*-pyridin-4-yl-benzenesulfonamide (**2d**), 4-[6′-(4- chlorophenyl)-4′-oxo-1′,4′-dihydro-3′*H*-spiro[cyclohexane-1,2′-thieno-[3,2-*d*]pyrimidin]-3′-yl]-*N*-(2-methyl-1,3-oxazol-4-yl)-benzenesulfonamide (**2e**).

Table I. Physical and analytical data of newly synthesized compounds

Compd.	Mol. formula (M_r)/solvent	M. p. (°C) Yield (%)	Analysis (calcd./found, %)			Compd.	Mol. formula (M_r)/solvent	M. p. (°C) Yield (%)	Analysis (calcd./found, %)		
			C	H	N				C	H	N
1	$C_{17}H_{16}ClNO_2S$ (333.8)/ethanol	150-152 79	61.16 61.13	4.83 4.81	4.19 4.16	**7a**	$C_{32}H_{27}ClN_2O_2S$ (539.08)/ethanol	154-156 66	71.29 71.26	5.05 5.01	5.19 5.16
2a	$C_{23}H_{22}ClN_3O_3S_2$ (488.0)/ethanol	205-207 68	56.60 56.58	4.54 4.53	8.61 8.59	**7b**	$C_{32}H_{26}Cl_2N_2O_2S$ (573.5)/ethanol	160-162 75	67.01 67.04	4.57 4.52	4.88 4.76
2b	$C_{24}H_{23}ClN_4O_3S_2$ (515.0)/isopropanol	235-237 60	55.96 55.94	4.50 4.49	10.87 10.84	**7c**	$C_{32}H_{26}ClFN_2O_2S$ (557.07)/ethanol	135-137 45	68.98 68.93	4.70 4.67	5.03 5.00
2c	$C_{27}H_{24}ClN_5O_3S_2$ (566.1)/dioxane	199-201 70	57.28 57.25	4.27 4.25	12.37 12.34	**7d**	$C_{34}H_{32}ClN_3O_2S$ (582.1)/ethanol	160-162 55	70.15 70.12	5.54 5.51	7.21 7.19
2d	$C_{28}H_{25}ClN_4O_3S_2$ (565.1)/isopropanol	220-222 59	59.50 59.48	4.46 4.44	9.91 9.90	**7e**	$C_{30}H_{25}ClN_2O_2S_2$ (545.1)/ethanol	155-157 51	66.09 66.11	4.62 4.59	5.14 5.09
2e	$C_{27}H_{25}ClN_4O_4S_2$ (569.1)/isopropanol	238-240 65	56.98 56.96	4.42 4.40	9.84 9.83	**8a**	$C_{32}H_{29}ClN_4OS$ (553.1)/ethanol	223-225 65	69.48 69.43	5.28 5.24	10.13 10.08
3	$C_{25}H_{23}ClN_2O_2S$ (450.9)/ethanol	181–183 70	66.58 66.56	5.14 5.13	6.21 6.19	**8b**	$C_{32}H_{28}Cl_2N_4OS$ (587.5)/ethanol	237-239 56	65.41 65.38	4.80 4.76	9.53 9.48
4a	$C_{25}H_{24}ClN_3O_3S_3$ (546.1)/isopropanol	180-182 68	54.98 54.96	4.42 4.40	7.69 7.66	**8c**	$C_{32}H_{28}ClFN_4OS$ (571.1)/ethanol	246-249 54	67.29 67.26	4.94 4.92	9.81 9.76
4b	$C_{30}H_{32}ClN_3O_3S_3$ (614.2)/dioxane	225-227 63	58.66 58.64	5.25 5.24	6.84 6.81	**8d**	$C_{34}H_{34}ClN_5OS$ (596.1)/isopropanol	209-211 52	68.50 68.47	5.75 5.73	11.75 11.69

Table I. continued

4c	$C_{30}H_{26}ClN_3O_3S_3$ (608.1)/dioxane	265-267 65	59.25 59.23	4.31 4.30	6.91 6.89	**8e**	$C_{30}H_{27}ClN_4OS_2$ (559.1)/isopropanol	190-192 60	64.44 64.39	4.86 4.82	10.02 10.07
5a	$C_{35}H_{28}ClN_5OS$ (602.1)/methanol	215-217 63	69.81 69.85	4.68 4.65	11.63 11.61	**9a**	$C_{32}H_{28}ClN_3O_2S$ (554.1)/ethanol	211-213 55	69.36 69.34	5.09 5.07	7.58 7.60
5b	$C_{35}H_{27}Cl_2N_5OS$ (636.5)/ethanol	225-227 80	66.04 66.07	4.27 4.25	11.00 10.91	**9b**	$C_{32}H_{27}Cl_2N_3O_2S$ (588.5)/ethanol	189-191 53	65.30 65.27	4.62 4.59	7.14 7.11
5c	$C_{35}H_{27}ClFN_5OS$ (620.1)/methanol	203-205 50	67.78 67.69	4.39 4.41	11.29 11.22	**9c**	$C_{32}H_{27}ClFN_3O_2S$ (572.1)/isopropanol	203-205 51	67.17 67.19	4.75 4.71	7.34 7.29
5d	$C_{37}H_{33}ClN_6OS$ (645.2)/ethanol	254-256 55	68.87 68.83	5.15 5.09	13.02 12.98	**9d**	$C_{34}H_{33}ClN_4O_2S$ (597.1)/ethanol	216-219 56	68.38 68.36	5.57 5.54	9.38 9.35
5e	$C_{33}H_{26}ClN_5OS_2$ (608.1)/dioxane	230-232 60	65.17 65.14	4.31 4.29	11.51 11.43	**9e**	$C_{30}H_{26}ClN_3O_2S_2$ (560.1)/isopropanol	232-234 60	64.32 64.29	4.68 4.65	7.50 7.45
6a	$C_{35}H_{27}ClN_4O_2S$ (603.1)/dioxane	270-272 68	69.70 69.66	4.51 4.50	9.29 9.27	**10a**	$C_{33}H_{27}ClN_4OS_2$ (595.1)/dioxane	215-217 56	66.59 66.56	4.57 4.54	9.41 9.38
6b	$C_{35}H_{26}Cl_2N_4O_2S$ (637.5)/ethanol	295-297 75	65.93 65.91	4.11 4.10	8.78 8.76	**10b**	$C_{33}H_{26}Cl_2N_4OS_2$ (629.6)/ethanol	195-197 51	62.95 62.92	4.16 4.18	8.90 8.87
6c	$C_{35}H_{26}ClFN_4O_2S$ (621.1)/ethanol	310-312 50	67.67 67.64	4.21 4.20	9.02 9.00	**10c**	$C_{33}H_{26}ClFN_4OS_2$ (613.1)/ethanol	168-170 55	64.64 64.61	4.27 4.25	9.13 9.11
6d	$C_{37}H_{32}ClN_5O_2S$ (646.2)/dioxane	320-322 60	68.76 68.73	4.99 4.97	10.83 10.81	**10d**	$C_{35}H_{32}ClN_5OS_2$ (638.2)/ethanol	205-207 65	65.86 65.83	5.05 5.03	10.97 10.93
6e	$C_{33}H_{25}ClN_4O_2S_2$ (609.1)/ethanol	281-283 55	65.06 65.04	4.13 4.12	9.19 9.16	**10e**	$C_{31}H_{25}ClN_4OS_3$ (601.2)/isopropanol	195-197 50	61.92 61.89	4.19 4.15	9.32 9.28

Table II. Spectral data of newly synthesized compounds

Compd.	IR (KBr) (ν_{max}, cm^{-1})	MS (*m*/*z*)	^1H, ^{13}C NMR (DMSO-d_6) (δ, ppm)
1	1712, 2885, 3032, 3425	MS 333 (M^+, 90 %)	2.53-3.05 (m, 10H, $5CH_2$), 7.47 (s, *J* = 3.6 Hz, 1H of thiophene ring), 7.58 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.80 (d, 2H, *J* = 8.2 Hz, Ar-H), 10.20 (brs, NH, D_2O exchangeable) 20.28, 20.67, 21.50, 22.69, 24.23, 25.58 ($5CH_2$), 84.56 (C-2 pyrimidine), 118.54-158.23 (10 sp^2 carbons), 172.5 (CO)
2a	1682, 2890, 3053, 3440	MS 488 (M^+, 78 %)	2.53-3.05 (m, 10H, $5CH_2$), 6.98-7.05 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.28-7.31 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.37 (H thiophene ring), 7.45 (d, 2H, *J* = 7.7 Hz, Ar-H), 8.02 (d, 2H, *J* = 7.6 Hz, Ar-H), 9.07 (br, 2H, NH_2), 10.40 (brs, NH)
2b	1316, 1678, 2900, 3053, 3471, 3343	MS 515 (M^+, 80 %)	2.42-3.00 (m, 10H, $5CH_2$), 6.94-7.11 (d, 2H, *J* = 8.3 Hz, Ar-H), 7.22-7.30 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.37 (1H thiophene ring), 7.78 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.95 (d, 2H, *J* = 8.2 Hz, Ar-H), 8.89 (br, 2H, NH_2), 10.40, 11.5 (brs, 2NH); 20.56, 21.23, 23.51, 24.12, 25.58 ($5CH_2$), 89.43 (*C*-2 pyrimidine), 119.56-159.73 (17 sp^2 C), 162.7 (CO)
2c	1331, 1685, 2893, 3034, 3400, 3349	MS 566 (M^+, 82 %)	2.48-3.06 (m, 10H, $5CH_2$), 7.01-7.15 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.28-7.31 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.47 (1H thiophene ring), 7.46 (d, 2H, *J* = 7.7 Hz, Ar-H), 7.62 (t, 1H, pyrimidine-H), 7.92 (d, 2H, *J* = 7.8 Hz, Ar-H), 8.21 (m, 2H, pyrimidine-H), 9.10, 10.25 (brs, 2NH, D_2O exchangeable) 21.06, 22.53, 23.64, 24.23, 26.38 ($5CH_2$), 89.63 (C-2 pyrimidine), 118.79-159.27 (20 sp^2 carbons), 162.5 (CO)
2d	1327, 1686, 3030, 3355, 3349	MS 565 (M^+, 72 %)	2.45-3.03 (m, 10H, $5CH_2$), 6.98-7.07 (d, 2H, *J* = 8.3 Hz, Ar-H), 7.28-7.36 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.40 (d, 1H, pyridine-H), 7.43 (d, 1H, pyridine-H), 7.49 (1H thiophene ring), 7.54 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.86 (d, 2H, *J* = 8.2 Hz, Ar-H), 8.35 (d, 1H, *J* = 2.01 Hz, pyridine-H), 8.42 (d, 1H, *J* = 2.0 Hz, pyridine-H), 9.35, 10.30, (brs, 2NH) 20.68, 22.23, 23.51, 24.01, 25.58 ($5CH_2$), 88.43 (C-2 pyrimidine), 118.62-159.56 (21 sp^2 carbons), 163.5 (CO)
2e	1689, 2931, 3471, 3343	MS 569 (M^+, 72 %)	2.43-3.01 (m, 10H, $5CH_2$), 6.91-7.01 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.28-7.32 (d, 2H, *J* = 8.3 Hz, Ar-H), 7.47 (s, 1H), 7.58 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.80 (d, 2H, *J* = 7.8 Hz, Ar-H), 8.10 (s, 1H, isoxazol ring), 9.25, 10.20 (brs, 2NH)
3	3423, 3053, 2930, 1705, 1681	MS 450 (M^+, 72 %)	2.43 (s, 3H, $COCH_3$), 2.53-3.05 (m, 10H, $5CH_2$), 6.98-7.07 (d, 2H, *J* = 8.3 Hz, Ar-H), 7.28-7.31 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.41 (s, 1H thiophene ring), 7.60 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.96 (d, 2H, *J* = 8.2 Hz, Ar-H), 10.23 (brs, NH); 20.73, 21.63, 22.31, 24.62. 26.27 ($5CH_2$), 26.80 (CH_3), 83.61 (C-2 pyrimidine), 119.19-158.72 (16 sp^2 carbons), 167.2 (CO, pyrimidine ring), 181.3 (CO, acetyl)
4a	1320, 1680, 3056, 3350,	MS 546 (M^+, 78 %)	2.19 (s, 3H, CH_3), 2.41-3.02 (m, 10H, $5CH_2$), 6.98-7.05 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.28-7.31 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.43 (s, 1H thiophene ring), 7.62 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.85 (d, 2H, *J* = 8.0 Hz, Ar-H), 9.52, 10.35 (brs, 2NH)
4b	1160, 1320, 1680, 3061, 33340, 3247	MS 614 (M^+, 78 %)	1.20-1.11 (m, 6H, cyclohexyl), 1.50-1.55 (m, 4H, cyclohexyl), 2.41-3.01 (m, 11H, $5CH_2$ $^+$CH), 6.97-7.04 (d, 2H, *J* = 8.3 Hz, Ar-H), 7.28-7.31 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.47 (s, 1H thiophene ring), 7.58 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.80 (d, 2H, *J* = 8.2 Hz, Ar-H), 9.25, 10.20 (brs, 2NH, D_2O exchangeable)

4c	1160, 1320, 1680, 3046, 33340, 3247	MS 608 (M^+, 78 %)	2.50-3.05 (m, 10H, $5CH_2$), 6.98-7.05 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.19-7.30 (m, 2H, Ar-H), 7.28-7.31 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.41 (s, 1H thiophene ring), 7.50-7.56 (m, 3H, Ar-H), 7.62 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.90 (d, 2H, *J* = 8.2 Hz, Ar-H), 9.25, 10.20 (brs, 2NH, D_2O exchangeable)
5a	3370, 3290, 3079, 2965, 1678, 1601	MS 602 (M^+, 72 %)	2.21-2.89 (m, 10H, $5CH_2$), 6.93-7.03 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.10-7.16 (m, 2H, Ar-H), 7.24-7.29 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.36 (s, 1H thiophene ring), 7.45-7.58 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.66 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.78–7.92 (m, 3H, Ar-H), 8.25 (s, 1H, pyridone ring), 9.40, 10.25 (s, 2NH, exchangeable with D_2O)
5b	3410, 3320, 3064, 2959, 1686, 1590	MS 636 (M^+, 72 %)	2.26-2.91 (m, 10H, $5CH_2$), 6.94-7.03 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.19-7.25 (d, 2H, *J* = 7.9 Hz, Ar-H), 7.29-7.36 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.41 (s, 1H thiophene ring), 7.48-7.58 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.65-7.80 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.83-7.92 (d, 2H, *J* = 8.00 Hz, Ar-H), 8.24 (s, 1H, pyridone ring), 9.80, 10.23 (s, 2NH)
5c	3405, 3280, 3056, 2973, 1678, 1601	MS 620 (M^+, 65 %)	2.34-2.98 (m, 10H, $5CH_2$), 6.99-7.07 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.13-7.21 (d, 2H, *J* = 7.8 *Hz*, Ar-H), 7.27-7.33 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.37 (s, 1H thiophene ring) 7.55-7.64 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.68-7.72 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.80-7.86 (d, 2H, *J* = 7.9 Hz, Ar-H), 8.19 (s, 1H, pyridone ring), 9.38, 10.21 (s, 2NH)
5d	3385, 3290, 3039, 2971, 1685, 1600	MS 645 (M^+, 72 %)	2.25-2.91 (m, 10H, $5CH_2$), 3.02 (2s, 6H, $2CH_3$), 6.95-7.01 (d, 2H, *J* = 8.3 Hz, Ar-H), 7.18-7.28 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.31-7.35 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.42 (s, 1H thiophene ring) 7.50-7.59 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.67-7.80 (d, 2H, *J* = 8.2, Ar-H), 7.89-7.93 (d, 2H, *J* = 8.3 Hz, Ar-H), 8.22 (s, 1H, pyridone ring), 9.95, 10.40 (s, 2NH)
5e	3370, 3280, 3053, 2951, 1682, 1595	MS 608 (M^+, 65 %)	2.32-2.94 (m, 10H, $5CH_2$), 6.98-7.06 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.13 (dd, *J* = 3.91, 5.06 Hz, 1H of thiophene), 7.24-7.30 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.36 (s, 1H thiophene ring), 7.40 (d, *J* = 3.5 Hz, 1H of thiophene), 7.46-7.59 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.63 (d, *J* = 5 Hz, 1H of thiophene), 7.71-7.80 (d, 2H, *J* = 8.2, Ar-H), 8.27 (s, 1H, pyridone ring), 9.52, 10.30 (s, 2NH, exchangeable with D_2O)
6a	3331, 3237, 3079, 1679, 1670, 2196	MS 603 (M^+, 75 %)	2.41-3.03 (m, 10H, $5CH_2$), 6.98-7.05 (d, 2H, *J* = 8.3 Hz, Ar-H), 7.13-7.18 (m, 2H, Ar-H), 7.28-7.31 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.40 (s, 1H thiophene ring), 7.46-7.58 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.80 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.84–8.00 (m, 3H, Ar-H), 8.20 (s, 1H, pyridone ring), 9.85, 10.36 (s, 2NH, exchangeable with D_2O)
6b	3401, 3339, 3079, 1683, 1676, 2204	MS 637 (M^+, 72 %)	2.53-3.05 (m, 10H, $5CH_2$), 6.96-7.02 (d, 2H, *J* = 8.3 Hz, Ar-H), 7.18-7.22 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.28-7.31 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.39 (s, 1H thiophene ring), 7.47-7.58 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.68-7.80 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.92 (d, 2H, *J* = 8.01 Hz, Ar-H), 8.24 (s, 1H, pyridone ring), 9.55, 10.25 (s, 2NH)
6c	3401, 3339, 3079, 1681, 1675, 2211	MS 621 (M^+, 72 %)	2.46-3.01 (m, 10H, $5CH_2$), 6.99-7.06 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.16-7.20 (d, 2H, *J* = 7.9 Hz, Ar-H), 7.28-7.31 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.40 (s, 1H thiophene), 7.58-7.62 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.64-7.71 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.83-7.88 (d, 2H, *J* = 7.8 Hz, Ar-H), 8.20 (s, H, pyridone), 9.65, 10.15 (s, 2NH)

6d	3420, 3380, 3059, 1686, 1674, 2212	MS 646 (M^+, 72 %)	2.46-3.01 (m, 10H, $5CH_2$), 3.05 (2s, 6H, $2CH_3$), 6.97-7.02 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.19-7.25 (d, 2H, *J* = 8.6 Hz, Ar-H), 7.29-7.31 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.41 (s, 1H thiophene ring), 7.52-7.61 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.80 (d, 2H, *J* = 8.2, Ar-H), 7.89-7.93 (d, 2H, *J* = 8.5 Hz, Ar-H), 8.21 (s, 1H, pyridone ring), 9.80, 10.32 (s, 2NH)
6e	3401, 3339, 3079, 1679, 1671, 2204	MS 609 (M^+, 72 %)	2.43-3.01 (m, 10H, $5CH_2$), 6.96-7.03 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.15 (d, *J* = 3.93, 5.05 Hz, 1H of thiophene), 7.28-7.31 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.39 (s, 1H thiophene ring), 7.43 (d, *J* = 3.6 Hz, 1H of thiophene), 7.48-7.58 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.64 (d, *J* = 5 Hz, 1H of thiophene), 7.69-7.80 (d, 2H, *J* = 8.2, Ar-H), 8.28 (s, 1H, pyridone ring), 9.55, 10.14 (s, 2NH, exchangeable with D_2O)
7a	3390, 3061, 1707, 1678	MS 539 (M^+, 63 %)	2.38-3.01 (m, 10H, $5CH_2$), 6.68–6.75 (dd, 2H, CH=CH), 6.93-7.01 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.11-7.19 (m, 2H, Ar-H), 7.26-7.30 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.39 (s, 1H thiophene), 7.50-7.59 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.65-7.72 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.89–8.00 (m, 3H, Ar-H), 9.45 (s, H, NH)
7b	3401, 3056, 1702, 1681	MS 573 (M^+, 68 %)	2.49-3.01 (m, 10H, $5CH_2$), 6.65–6.71 (d,d, 2H, CH=CH), 6.99-7.06 (d, 2H, *J* = 8.3 Hz, Ar-H), 7.20-7.26 (d, 2H, *J* = 7.9 Hz, Ar-H), 7.31-7.34 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.40 (s, 1H thiophene ring), 7.49-7.61 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.69-7.83 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.88-7.93 (d, 2H, *J* = 8.01 Hz, Ar-H), 9.60 (s, H, NH)
7c	3401, 3049, 1705, 1678	MS 557 (M^+, 72 %)	2.50-3.01 (m, 10H, $5CH_2$), 6.65–6.72 (dd, 2H, CH=CH), 7.01-7.08 (d, 2H, *J* = 8.3 Hz, Ar-H), 7.18-7.21 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.25-7.30 (d, 2H, *J* = 8.3 Hz, Ar-H), 7.36 (s, 1H thiophene ring), 7.48-7.60 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.67-7.75 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.82-7.89 (d, 2H, *J* = 7.9 Hz, Ar-H), 9.25 (s, H, NH)
7d	3420, 3049, 1712, 1678	MS 582 (M^+, 53 %)	2.49-3.03 (m, 10H, $5CH_2$), 3.08 (2s, 6H, $2CH_3$), 6.71-6.80 (dd, 2H, CH=CH), 6.99-7.06 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.20-7.27 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.30-7.33 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.43 (s, 1H thiophene ring), 7.56-7.63 (d, 2H, *J* = 8.3 Hz, Ar-H), 7.68-7.80 (d, 2H, *J* = 8.2, Ar-H), 7.88-7.94 (d, 2H, *J* = 8.4 Hz, Ar-H), 9.93 (s,NH)
7e	3370, 3079, 1705, 1678	MS 545 (M^+, 72 %)	2.39-3.00 (m, 10H, $5CH_2$), 6.65–6.72 (dd, 2H, CH=CH), 6.97-7.04 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.15 (dd, *J* = 3.92, 5.06 Hz, 1H of thiophene), 7.23-7.30 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.37 (s, 1H thiophene ring), 7.41 (d, *J* = 3.8 Hz, 1H of thiophene), 7.46-7.56 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.63 (d, *J* = 4.9 Hz, 1H of thiophene), 7.70-7.78 (d, 2H, *J* = 8.2, Ar-H), 9.05 (s, NH)
8a	3410, 3079, 1679	MS 553 (M^+, 43 %)	2.29-3.04 (m, 12H, $6CH_2$), 3.44 (m, 1H, pyrazole-H5), 7.01-7.08 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.15-7.19 (m, 2H, Ar-H), 7.26-7.30 (d, 2H, *J* = 8.3 Hz, Ar-H), 7.42 (s, 1H thiophene ring), 7.46-7.53 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.63-7.76 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.81-7.89 (m, 3H, Ar-H), 9.32, 10.14 (s, 2H,2NH)
8b	3408, 3390, 3058, 1680	MS 587 (M^+, 39 %)	2.38-3.02 (m, 12H, $6CH_2$), 3.39 (m, 1H, pyrazole-H5), 6.93-7.00 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.12-7.18 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.25-7.30 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.35 (s, 1H thiophene ring), 7.41-7.50 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.62-7.68 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.83-7.91 (d, 2H, *J* = 8.01 Hz, Ar-H), 9.10, 10.05 (s, 2H, 2NH)

8c	3395, 3345, 3046, 1675	MS 571 (M^+, 41 %)	2.16-3.01 (m, 12H, $6CH_2$), 3.42 (m, 1H, pyrazole-H5), 6.94-7.01(d, 2H, *J* = 8.3 Hz, Ar-H), 7.11-7.15 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.20-7.28 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.41 (s, 1H thiophene ring), 7.53-7.60 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.67-7.72 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.82-7.86 (d, 2H, *J* = 7.9 Hz, Ar-H), 9.15, 10.25 (s, 2H, 2NH)
8d	3403, 3395, 3043, 1683	MS 596 (M^+, 35 %)	2.16-3.06 (m, 12H, $6CH_2$), 3.12 (2s, 6H, $2CH_3$), 3.49 (m, 1H, pyrazole-H5), 6.94-7.01 (d, 2H, *J* = 8.3 Hz, Ar-H), 7.16-7.23 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.30-7.36 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.42 (s, 1H thiophene ring), 7.53-7.65 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.69-7.81 (d, 2H, *J* = 8.2, Ar-H), 7.87-7.96 (d, 2H, *J* = 8.4 Hz, Ar-H), 9.55, 10.25 (s, 2H, 2NH)
8e	3410, 3365, 3049, 1679	MS 559 (M^+, 36 %)	2.19-3.00 (m, 12H, $6CH_2$), 3.51 (m, 1H, pyrazole-H5), 6.98-7.02 (d, 2H, *J* = 8.3 Hz, Ar-H), 7.14 (dd, *J* = 3.93, 5.06 Hz, 1H of thiophene), 7.26-7.30 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.36 (s, 1H thiophene ring), 7.44 (d, *J* = 3.5 Hz, 1H of thiophene), 7.44-7.52 (d, 2H, Ar-H), 7.63 (d, 1H of thiophene), 7.67-7.80 (d, 2H, *J* = 8.3, Ar-H), 9.35, 10.32 (s, 2H, 2NH)
9a	3400, 3056, 1678	MS 554 (M^+, 40 %)	2.21-3.04 (m, 12H, $6CH_2$), 3.64 (m, 1H, pyrazole-H5), 7.00-7.07 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.13-7.19 (m, 2H, Ar-H), 7.25-7.30 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.43 (s, 1H thiophene ring), 7.48-7.52 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.64-7.78 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.83-7.89 (m, 3H, Ar-H), 9.50 (br, NH)
9b	3394, 3049, 1681	MS 588 (M^+, 37 %)	2.19-3.01 (m, 12H, $6CH_2$), 3.72 (m, 1H, pyrazole-H5), 6.94-7.01 (d, 2H, *J* = 7.9 Hz, Ar-H), 7.15-7.19 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.27-7.31 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.39 (s, 1H thiophene ring), 7.45-7.52 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.63-7.69 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.85-7.94 (d, 2H, *J* = 8.1 Hz, Ar-H), 9.28 (br, NH)
9c	3345, 3049, 1678	MS 572 (M^+, 42 %)	2.14-3.01 (m, 12H, $6CH_2$), 3.69 (m, 1H, pyrazole-H5), 6.98-7.04 (d, 2H, *J* = 8.3 Hz, Ar-H), 7.13-7.19 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.23-7.31 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.43 (s, 1H thiophene ring), 7.54-7.62 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.68-7.76 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.83-7.88 (d, 2H, *J* = 8.0 Hz, Ar-H), 9.33 (br, NH)
9d	3351, 3042,1674	MS 597 (M^+, 41 %)	2.10-3.02 (m, 12H, $6CH_2$), 3.19 (2s, 6H, $2CH_3$), 3.67 (m, 1H, pyrazole-H5), 6.98-7.04 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.18-7.25 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.32-7.38 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.45 (s, 1H thiophene ring), 7.57-7.66 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.70-7.82 (d, 2H, *J* = 8.1, Ar-H), 7.88-7.97 (d, 2H, *J* = 8.3 Hz, Ar-H), 9.27 (br, NH)
9e	3385, 3053, 1682	MS 560 (M^+, 29 %)	2.21-3.02 (m, 12H, $6CH_2$), 3.76 (m, 1H, pyrazole-H5), 6.96-7.01 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.16 (dd, *J* = 3.91, 5.07 Hz, 1H of thiophene), 7.28-7.33 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.39 (s, 1H thiophene ring), 7.43 (d, *J* = 3.7 Hz, 1H of thiophene), 7.49-7.56 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.72 (d, *J* = 4.9 Hz, 1H of thiophene), 7.78-7.84 (d, 2H, *J* = 8.2, Ar-H), 9.42 (br, NH)
10a	3410, 3385, 3053, 1682	MS 595 (M^+, 42 %)	2.19-3.04 (m, 10H, $5CH_2$), 6.89-7.01 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.09-7.18 (m, 2H, Ar-H), 7.22-7.28 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.38 (s, 1H thiophene ring), 7.42-7.49 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.56-7.68 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.82-7.89 (m, 3H, Ar-H), 8.23 (s, 1H, pyrimidine), 9.28, 10.05 (brs, 2NH, exchangeable with D_2O);

10b	3416, 3369, 3050, 1680	MS 629 (M^+, 36 %)	2.26-3.03 (m, 10H, $5CH_2$), 6.99-7.05 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.19-7.23 (d, 2H, *J* = 7.9 Hz, Ar-H), 7.29-7.33 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.37 (s, 1H thiophene ring), 7.42-7.48 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.61-7.67 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.81-7.92 (d, 2H, *J* = 8.0 Hz, Ar-H), 8.31 (s, 1H, pyrimidine), 9.28, 10.24 (brs, 2NH)
10c	3412, 3376, 3063, 1683	MS 613 (M^+, 42 %)	2.19-3.04 (m, 10H, $5CH_2$), 7.01-7.09 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.15-7.21 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.25-7.32 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.40 (s, 1H thiophene ring), 7.47-7.56 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.63-7.69 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.81-7.87 (d, 2H, *J* = 8.0 Hz, Ar-H), 8.35 (s, 1H, pyrimidine), 9.12, 10.15 (brs, 2NH)
10d	3425, 3351, 3050, 1679	MS 638 (M^+, 37 %)	2.13-3.03 (m, 10H, $5CH_2$), 3.19 (2s, 6H, $2CH_3$), 7.01-7.09 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.16-7.27 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.30-7.37 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.43 (s, 1H thiophene ring), 7.53-7.62 (d, 2H, *J* = 7.9 Hz, Ar-H), 7.68-7.80 (d, 2H, *J* = 8.1, Ar-H), 7.86-7.99 (d, 2H, Ar-H), 8.35 (s, 1H, pyrimidine), 9.18, 10.36 (brs, 2NH, exchangeable with D_2O)
10e	3431, 3385, 3061, 1681	MS 601 (M^+, 32 %)	2.24-3.06 (m, 10H, $5CH_2$), 6.99-7.06 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.18 (dd, *J* = 3.87, 5.03 Hz, 1H of thiophene), 7.30-7.36 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.33 (s, 1H thiophene ring), 7.38 (d, *J* = 3.6 Hz, 1H of thiophene), 7.47-7.54 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.68 (d, *J* = 5.0 Hz, 1H of thiophene), 7.82-7.87 (d, 2H, Ar-H),8.30 (s, 1H, pyrimidine), 9.34, 10.26 (brs, 2NH)

*3′-(4-Acetylphenyl)-6′-(4-chlorophenyl)-1′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidine-4′(3′H)-one* (**3**). – A mixture of **1** (3.33 g, 0.01 mol) and 4-aminoacetophenone (1.35 g, 0.01 mol) in glacial acetic acid (20 mL) was refluxed for 6 h in the presence of sodium acetate (1.64 g, 0.02 mol). The reaction product was poured onto ice/water and neutralized with diluted NaOH. The obtained product was filtered off and washed with water to give pale yellow crystals.

N-*({4-[6′-(4-chlorophenyl)-4′-oxo-1′,4′-dihydro-3′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]-pyrimidin-3′-yl]phenyl}sulfonyl) carbothioamide* (**4*a*-*c***). *General procedure.* – A mixture of **2a** (2.44 g, 0.05 mol) and anhydrous potassium carbonate (1.38 g, 0.01 mol) in dry acetone (50 mL) was refluxed under continuous stirring for 1.5 h. While hot, a solution of the appropriate isothiocyanate derivative, namely, methylisothiocyanate, cyclohexyl-isothiocyanate and phenyl isothiocyanate (0. 75 mol), in dry acetone was added dropwise and refluxing was continued for a further 18 h. Excess acetone was removed under reduced pressure and the obtained solid residue was washed with water, filtered and recrystallized from the proper solvent to give **4a-c**, respectively. The following compounds were prepared: *N*-({4-[6′-(4-chlorophenyl)-4′-oxo-1′,4′-dihydro-3′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-3′-yl]-phenyl}sulfonyl)methyl-thioamide (**4a**), *N*-({4-[6′-(4-chlorophenyl)-4′-oxo-1′,4′-dihydro-3′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-3′-yl]phenyl}sulfonyl)cyclohexyl-carbothioamide (**4b**), *N*-({4-[6′-(4-chlorophenyl)-4′-oxo-1′,4′-dihydro-3′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*] pyrimidin]-3′-yl]phenyl}sulfonyl)-phenyl-carbothioamide (**4c**).

*6-{4-[6′-(4-Chlorophenyl)-4′-oxo-1′,4′-dihydro-3′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]-pyrimidin]-3′-yl]phenyl}-2-imino-4-aryl-1,2-dihydropyridine-3-carbonitriles* (**5*a*-*e***). *General procedure.* – A mixture of acetyl derivative **3** (4.51 g, 0.01 mol), malononitrile (0.66 g, 0.01 mol) and the appropriate aldehyde (0.01 mol), and ammonium acetate (6.16 g, 0.08 mol) in *n*-butanol (50 mL) was heated under reflux for 8 h. The solid that separated upon cooling was filtered off, washed with water and recrystallized from the proper solvent to obtain **5a-e**, respectively. The following compounds were prepared: 6-{4-[6′-(4-chlorophenyl)-4′-oxo-1′,4′-dihydro-3′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-3′-yl]phenyl}-2-imino-4-phenyl-1,2-dihydropyridine-3-carbonitrile (**5a**), 4-(4-chlorophenyl)-6-{4-[6′-(4-chlorophenyl)-4′-oxo-1′,4′-dihydro-3′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-3′-yl]phenyl}-2-imino-1,2-dihydropyridine-3-carbonitrile (**5b**), 6-{4-[6′-(4-chlorophenyl)-4′-oxo-1′,4′-dihydro-3′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-3′-yl]phenyl}-4-(4-fluorophenyl)-2-imino-1,2-dihydropyridine-3-carbonitrile (**5c**).

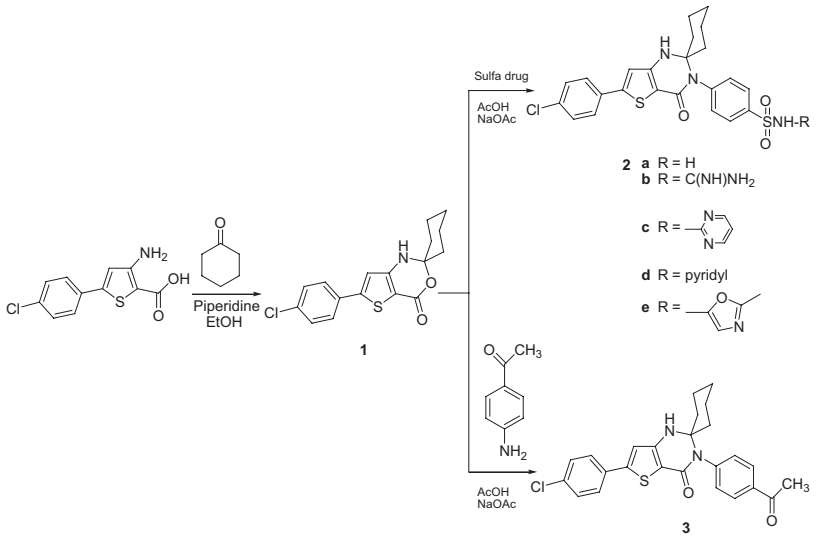
*6-{4-[6′-(4-Chlorophenyl)-4′-oxo-1′,4′-dihydro-3′*H*-spiro[cyclohexane-1,2′-thieno-[3,2-*d*]-3′-yl]-pyrimidin]-2-oxo-4-substituted aryl-1,2-dihydropyridine-3-carbonitriles* (**6*a*-*e***). *General procedure.* – A mixture of acetyl derivative **3** (4.51 g, 0.01 mol), ethyl cyanoacetate (1.13 g, 0.01 mol) and the appropriate aldehyde (0.01 mol), and ammonium acetate (6.16 g, 0.08 mol) in *n*-butanol (50 mL) was heated under reflux for 8 h. The solid that separated upon cooling was filtered off, washed with water, recrystallized from the proper solvent to give **6a-e**, respectively. The following compounds were prepared: 6-{4-[6′-(4-chlorophenyl)-4′-oxo-1′,4′-dihydro-3′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-3′-yl]phenyl}-2-oxo-4-phenyl-1,2-dihydropyridine-3-carbonitrile (**6a**), 4-(4-chlorophenyl)-6-{4-[6′-(4-chlorophenyl)-4′-oxo-1′,4′-dihydro-3′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-3′-yl]phenyl}-2-oxo-1,2-dihydropyridine-3-carbonitrile (**6b**), 6-{4-[6′-(4-chlorophenyl)-4′-oxo-1′,4′-dihydro-3′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-3′-yl]phenyl}-4-(4-flurophenyl)-2-

oxo-1,2-dihydropyridine-3-carbonitrile (**6c**), 6-{4-[6′-(4-chlorophenyl)-4′-oxo-1′,4′-dihydro-3′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-3′-yl]phenyl}-4-(4-dimethylamino) phenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (**6d**), 6-{4-[6′-(4-chlorophenyl)-4′-oxo-1′,4′-dihydro-3′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-3′-yl]phenyl}-2-oxo-4-(2-thienyl)-1,2-dihydropyridine-3-carbonitrile (**6e**).

*6′-(4-Clorophenyl)-3′-{4-[(2Z)-3-substituted arylprop-2-enoyl]phenyl}-1′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-4′(3′H)-ones (**7a-e**). General procedure*. – A mixture of acetyl derivative **3** (4.51 g, 0.01 mol) and the appropriate aldehyde (0.01 mol) in 5 % ethanolic sodium hydroxide solution (40 mL) was refluxed under stirring for 18 h. The reaction mixture was poured onto ice/cold water and neutralized with dil. HCl. The formed precipitate was filtered off and recrystallized from the proper solvent to give **7a-e**, respectively. The following compounds were prepared: 6′-(4-clorophenyl)-3′-{4-[(2Z)-3-phenylprop-2-enoyl]phenyl}-1′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-4′(3′H)-one (**7a**), 6′-(4-clorophenyl)-3′-{4-[(2Z)-3-(4-chlorophenyl)-prop-2-enoyl]phenyl}-1′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*] pyrimidin]-4′(3′*H*)-one (**7b**), 6′-(4-clorophenyl)-3′-{4-[(2Z)-3-(4-florophenyl)prop-2-enoyl] phenyl}-1′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-4′(3′*H*)-one (**7c**), 6′-(4-clorophenyl) -3′-(4-{(2Z)-3-[4-(dimethylamino)-phenyl]prop-2-enoyl]-phenyl}-1′*H*-spiro-[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-4′(3′*H*)-one (**7d**), 6′-(4-clorophenyl)-3′-(4-[(2Z)-3-[2-(thienyl)prop-2-enoyl]phenyl}-1′*H*-spiro [cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-4′(3′*H*)-one (**7e**).

*6′-(4-Chlorophenyl)-3′-[4-(5-substituted aryl-4,5-dihydro-1*H*-pyrazol-3-yl)phenyl]-1′*H*-spiro-[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-4′(3′*H*)-ones (**8a-e**). General procedure*. – A mixture of unsaturated ketone **7a-e** (0.01 mol) and 98 % hydrazine hydrate in absolute ethanol (2 mL) was refluxed for 6 h. Upon cooling, white crystals were obtained, filtered off and recrystallized from the proper solvent to obtain **8a-e**, respectively. The following compounds were prepared: 6′-(4-chlorophenyl)-3′-[4-(5-phenyl-4,5-dihydro-1*H*-pyrazol-3yl)phenyl]-1′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]-pyrimidin]-4′(3′*H*)-one (**8a**), 6′-(4-chloro phenyl)-3′-{4-(5-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-3yl)phenyl]-1′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-4′(3′*H*)-one (**8b**), 6′-(4-chlorophenyl)-3′-{4-(5-(4-florophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)-phenyl]-1′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*] pyrimidin]-4′(3′*H*)-one (**8c**), 6′-(4-chlorophenyl)-3′-(4-{5-[4-(dimethylamino)phenyl]-4,5-dihydro-1*H*-pyrazol-3-yl)phenyl]-1′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-4′(3′*H*)-one (**8d**), 6′-(4-chlorophenyl)-3′-{4-[5-(2-thienyl)-4,5-dihydro-1*H*-pyrazol-3yl)phenyl]-1′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-4′(3′H)-one (**8e**).

*6′-(4-Chlorophenyl)-3′-[4-(5-substituted aryl-4,5-dihydroisoxazol-3-yl)phenyl]-1′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-4′(3′H)-ones (**9a-e**). General procedure*. – A mixture of unsaturated ketone **7a-e** (0.01 mol) and hydroxylamine hydrochloride (1.38 g, 0.02 mol) in sodium hydroxide solution (0.5 g NaOH in 0.5 mL water) in ethanol (60 mL) was refluxed for 3 h. The product obtained upon cooling was filtered off, washed with water and recrystallized from the proper solvent to obtain the desired **9a-e**, respectively. The following compounds were prepared: 6′-(4-chlorophenyl)-3′-[4-(5-phenyl-4,5-dihydroisoxazol-3-yl) phenyl]-1′*H*-spiro[cyclohexane-1, 2′-thieno[3,2-*d*]pyrimidin]-4′(3′*H*)-one (**9a**), 6′-(4-chlorophenyl)-3′-{4-[5-(4-chlorophenyl)-4,5-dihydroisoxazol-3-yl]phenyl}-1′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-4′(3′*H*)-one (**9b**), 6′-(4-chlorophenyl)-3′-{4-[5-(4-fluorophenyl)-4,5-dihydroisoxazol-3-yl]phenyl}-1′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-4′(3′*H*)-one (**9c**), 6′-(4-chlorophenyl)-3′-(4-{5-[4-(dimethylamino)phenyl]-4,5-dihydroisoxa-



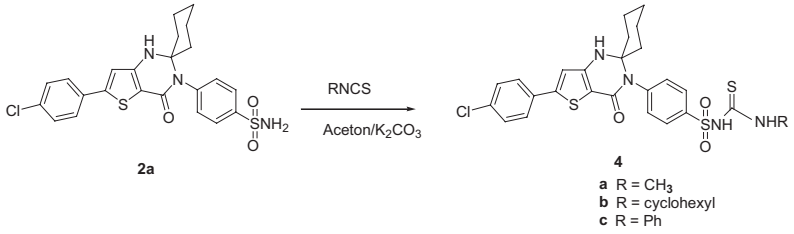
Scheme 1

zol-3-yl]phenyl}-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin-4′(3′*H*)-one (**9d**), 6′-(4-chlorophenyl)-3′-{4-{5-(2-thienyl)-4,5-dihydro-isoxazol-3-yl]phenyl}-1′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-4′(3′H)-one (**9e**).

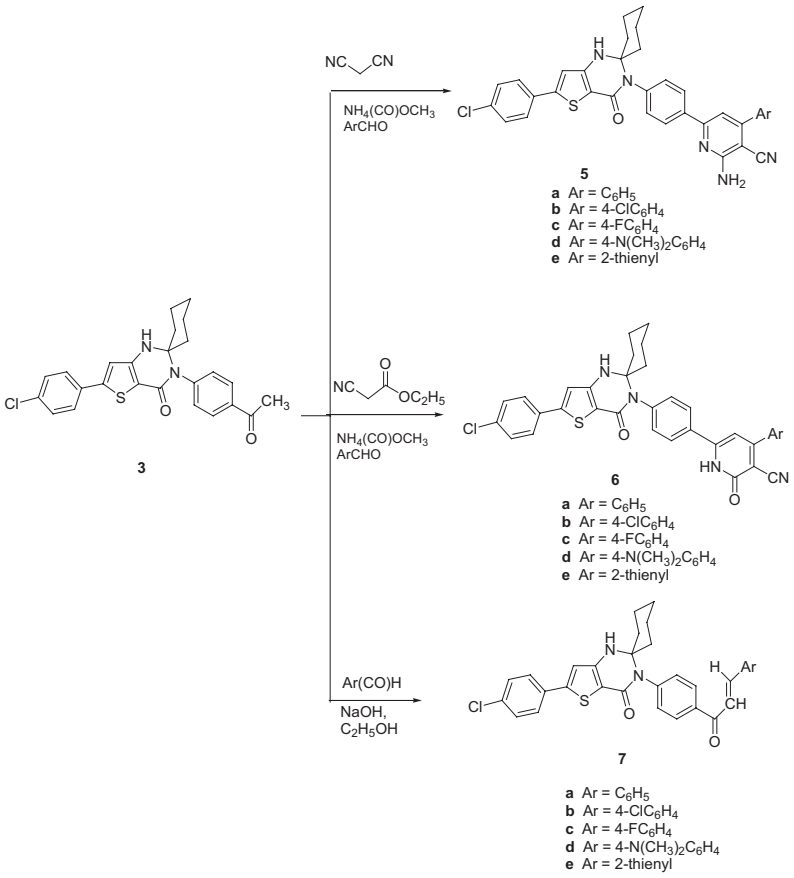
*6′-(4-Chlorophenyl)-3′-[4-(6-aryl(substituted)-2-thioxo-1,2-dihydropyrimidin-4-yl)-phenyl]-1′*H*-spiro[cyclohexane-1,2′-thieno-[3,2-*d*]pyrimidin]-4′(3′H)-ones (**10a-e**). General procedure.* – A mixture of unsaturated ketone **7a-e** (0.01 mol), thiourea (0.01 mol) and sodium hydroxide (0.1 g) in absolute ethanol (30 mL) was refluxed for 6 h. The reaction mixture was concentrated under vacuum, cooled and neutralized with dilute HCl. The formed product was filtered off and washed with water to yield **10a-e**, respectively. The following compounds were prepared: 6′-(4-chlorophenyl)-3′-[4-(6-phenyl-2-thioxo-1,2-dihydropyrimidin-4-yl)-phenyl]-1′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-4′(3′*H*)-one (**10a**), 6′-(4-chlorophenyl)-3′-{4-[6-(4-chlorophenyl)-2-thioxo-1,2-dihydropyrimidin-4-yl]phenyl}-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-4′(3′*H*)-one (**10b**), 6′-(4-chlorophenyl)-3′-{4-[6-(4-fluorophenyl)-2-thioxo-1,2-dihydropyrimidin-4-yl]phenyl}-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-4′(3′H)-one (**10c**), 6′-(4-chlorophenyl)-3′-(4-{6-[4-(dimethylamino) phenyl]-2-thioxo-1,2-dihydropyrimidin-4-yl}-phenyl)-1′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]-pyrimidin]-4′(3′*H*)-one (**10d**), 6′-(4-chlorophenyl)-3′-{4-[6-(2-thienyl)-2-thioxo-1,2-dihydropyrimidin-4-yl]phenyl}-1′*H*-spiro[cyclohexane-1,2′-thieno[3,2-*d*]pyrimidin]-4′(3′*H*)-one (**10e**).

Biological screening

Antimicrobial activity. – Antimicrobial activity of the newly synthesized compounds was tested *in vitro* against the following bacteria: Gram-negative bacteria *Escherichia coli* (ATCC 23556), *Klebsiella pneumoniae* (ATCC 27853) and *Pseudomonas aeruginosa* (ATCC 10145), Gram-

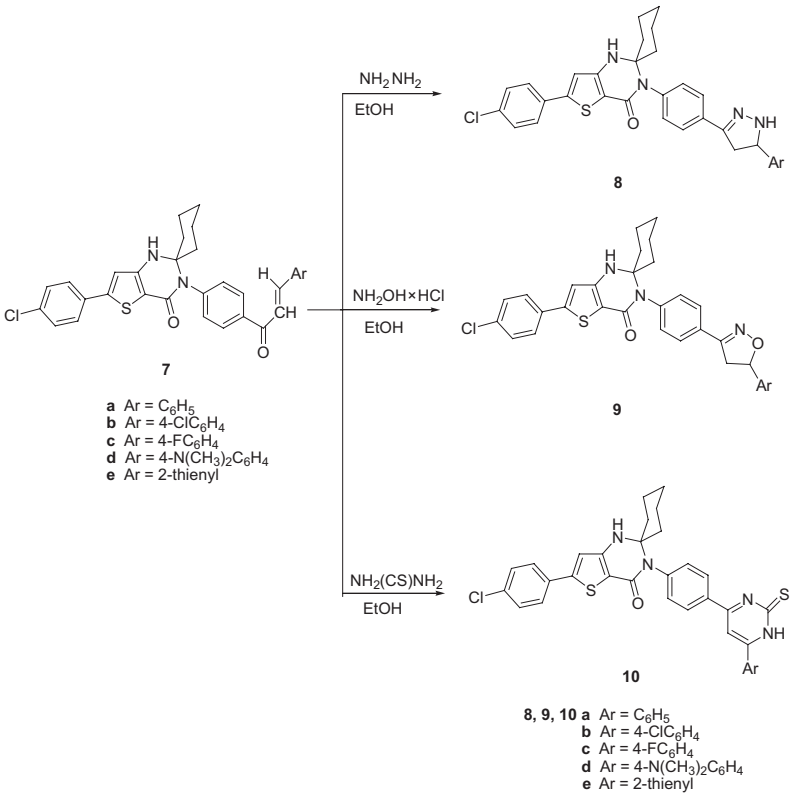


Scheme 2



Scheme 3

-positive bacteria *Streptococcus lactis* (ATCC 6523), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 5787) and the fungi *Candida albicans* (ATCC 15056), *Aspergillus flavus* (ATCC-24556) and *Ganoderma lucidum* (ATCC-96918). All microorganisms were purchased from the American Type Culture Collection (Manassas, VA, USA). The newly synthesized



Scheme 4

compounds were dissolved in dimethylsulfoxide (DMSO) and tested for antimicrobial activity by the agar disk diffusion technique (24), taking ciprofloxacin (25) and ketoconazole (26) as reference drugs for bacteria and fungi, respectively. A solution of 100 μg mL^{-1} of the test compound was applied and microplate-wells, 1 cm in diameter, were used. Zones of inhibition were measured with calipers or automated scanners and were compared with those of the standards. Ciprofloxacin (0.15 μmol mL^{-1}) and ketoconazole (0.037 μmol mL^{-1}) were used as reference drugs for antibacterial and antifungal activity, respectively. Compound-impregnated disks were placed on an agar plate containing a standard suspension of microorganisms. The plate was incubated for 24 h at 37 °C.

For evaluation of minimum inhibitory concentration (*MIC*) by the serial plate dilution method (27), 5 mg of each test compounds were dissolved in 1 mL of DMSO separately to prepare stock solutions. Serial dilutions were prepared from each stock solution. The plates were incubated at 37 °C for 24 h. *MIC* is defined as the lowest concentration (μmol mL^{-1}) of the test compound that results in no visible growth on the plates. DMSO was used as a solvent control to ensure that the solvent had no effect on bacterial growth. The results are summarized in Tables III and IV.

RESULTS AND DISCUSSION

Chemistry

The first step of our strategy was focused on ring transformation of oxazin to thieno[3,2-*d*]pyrimidine (28, 29). Treatment of 3-amino-5-(4-chlorophenyl)thiophene-2-carboxylic acid with cyclohexanone yielded 6′-(4-chlorophenyl)-spiro-[cyclohexane-1,2′-thieno[3,2-*d*][1,3]-oxazin]-4′(1′*H*)-one (**1**) in good yield (Scheme 1).

Transformation of oxazine derivative ring into *N*-substituted aryl sulfonamide derivative was carried out through the treatment of 1,3-oxazine (*i. e.*, thieno[3,2-*d*][1,3]oxazin--4-one, **1**) with sulfa drug, namely, sulfanilamide, sulfaguanidine, sulfadiazine, sulfapyridine or sulfamethoxazole, in refluxing glacial acetic acid and in the presence of anhydrous sodium acetate, yielding the title compounds **2a-e** in a moderate yield.

Variation of different aryl or heterocyclic moieties in 2/3 position of thieno[3,2-*d*]-[1,3] oxazin-4-one (**1**) may influence the pharamacological action. Herein, we explore the influence of aryl or heteroaryl moieties in *N2* of pyrimidine nucleus. 1,3-Oxazine (**1**) was allowed to condense with *p*-amino-acetophenone in glacial acetic acid in the presence of anhydrous sodium acetate to give the corresponding key intermediate, thieno[3,2-*d*]-pyrimidin-one (**3**) in a good yield (Scheme 1).

Free amino groups of sulfonyl derivatives **2a** reacted with methyl isothiocyanate derivatives in dry acetone and anhydrous potassium carbonate, affording thiourea derivatives **4a-c** (Scheme 2). Besides correct values of elemental analyses, IR, NMR and mass spectra of compounds **4a-c** are in agreement with the assigned structures (Tables I, II). IR spectrum of **4a** shows C=O at δ 1680 cm^{-1} and broad absorption bands at δ 3340 and 3325 cm^{-1} for three NH, respectively. ^{1}H NMR spectra showed singlet CH_3 at δ 2.19 ppm, multiplet signals for 5 CH_2 at δ 2.41 to 3.02 ppm, singlet signal for the thiophene proton at δ 7.43 ppm and four duplet signals at δ 6.98–7.05 (d, 2H, J = 8.5 Hz, Ar-H), δ 7.28–7.31 (d, 2H, J = 8.2 Hz, Ar-H), δ 7.62 (d, 2H, J = 8.0 Hz, Ar-H), δ 7.85 ppm (d, 2H, J = 8.0 Hz, Ar-H) for two 4-substituted phenyls. Moreover, broad absorption signals for three NH groups at δ 9.52–10.35 ppm were observed, respectively. The mass spectra of all compounds showed corresponding molecular ion (M^+) in addition to characteristic fragmentation peaks. Chloro-substituted compounds displayed M^{+2} peaks in addition to the molecular ion peaks (M^+).

Refluxing compound **3** with the appropriate aldehyde and malononitrile or ethyl cyanoacetate in the presence of excess ammonium acetate in *n*-butanol afforded the corresponding aminopyridine **5a-e** and pyridone derivatives **6a-e**, respectively. Also, treatment of acetyl derivative **3** with appropriate aldehydes in 5 % ethanolic sodium hydroxide solution, afforded the corresponding α,β-unsaturated ketones **7a-e** in moderate yields (Scheme 3). The ^{1}H NMR spectra of compounds **5**, **6** and **7** revealed absorption bands of five (CH_2–) methylene groups as multiplet absorption signals of the cyclohexane ring around δ 2.46–3.01 ppm (Table II).

α,β-unsaturated ketone is a simple substrate to synthesize a variety of heterocycles of known biological interest. Cyclocondensation of compounds **7a-e** with *N*-nucleophiles under appropriate experimental conditions afforded pyrazoline derivatives **8a-e**, isoxazolines **9a-e** and thiopyrimidine derivatives **10a-e** (Scheme 4).

Postulated structures of the newly synthesized compounds **8**, **9** and **10** are in agreement with their IR, NMR spectral and elemental analysis data (Tables I, II). The ^{1}H NMR

Table III. Minimal inhibitory concentration of the synthesized compounds against bacteria

Compd.	*MIC* (μmol L^{-1})					
	Gram-negative bacteria			Gram-positive bacteria		
	Escherichia coli	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Streptococcus lactis*	*Staphylococcus aureus*	*Enterococcus faecalis*
1	8	8	7	6	5	8
2a	5	3	5	4	4	5
2b	6	4	7	3	4	5
2c	3	3	2	2	1	2
2d	6	6	7	3	3	4
2e	2	3	2	2	2	4
3	7	7	5	5	4	7
4a	6	5	5	5	4	6
4b	6	5	5	5	5	7
4c	7	6	6	5	4	5
5a	7	6	7	4	5	6
5b	4	4	4	1	2	3
5c	4	3	3	2	2	4
5d	6	7	7	5	4	6
5e	8	6	7	4	4	7
6a	9	7	6	5	5	7
6b	8	7	6	5	7	6
6c	6	9	7	5	4	6
6d	7	8	6	6	6	7
6e	8	9	6	8	7	9
7a	7	8	7	8	6	8
7b	9	8	9	6	7	9
7c	7	9	7	8	8	9
7d	8	6	8	-	10	-
7e	9	8	8	6	6	7
8a	8	9	7	5	6	6
8b	3	2	3	2	1	2
8c	2	3	4	1	1	2
8d	9	7	7	5	6	8
8e	6	6	5	4	5	5
9a	10	7	7	5	5	7
9b	4	4	3	2	1	3
9c	3	3	2	3	2	4
9d	8	7	6	6	5	7
9e	7	6	5	5	4	5
10a	7	8	8	5	5	7
10b	2	1	4	1	2	3
10c	4	2	2	2	3	3
10d	9	8	7	4	7	7
10e	6	6	5	3	2	5
Negative control	NI	NI	NI	NI	NI	NI
Cipro-floxacin	5	4	4	2	2	4

DMSO is a negative control; also the solvent for test compounds and the reference drug.
NI – No inhibition

Table IV. Minimal inhibitory concentration of the synthesized compounds against fungi

Compd.	*MIC* (μmol mL^{-1})		
	Candida albicans	*Aspergillus flavus*	*Ganoderma lucidum*
1	7	6	5
2a	10	12	11
2b	13	11	10
2c	11	12	12
2d	13	-	-
2e	12	10	10
3	7	6	7
4a	-	16	-
4b	14	12	12
4c	11	10	12
5a	7	7	6
5b	6	7	6
5c	6	6	7
5d	8	6	7
5e	8	11	10
6a	9	9	8
6b	9	8	10
6c	7	8	8
6d	-	10	-
6e	8	9	9
7a	7	6	8
7b	8	8	7
7c	8	6	7
7d	6	7	6
7e	6	8	7
8a	5	5	6
8b	4	5	4
8c	2	2	1
8d	7	6	6
8e	3	2	2
9a	7	4	6
9b	6	6	8
9c	6	5	7
9d	8	6	6
9e	6	5	7
10a	8	6	6
10b	5	4	4
10c	4	3	3
10d	7	6	7
10e	4	4	5
Negative control	NI	NI	NI
Ketoconazole	3	2	3

DMSO is a negative control, also the solvent for test compounds and the reference drug.
NI – No inhibition

spectrum of **8c**, for example, showed multiple signals at δ 2.16 to 3.01 ppm corresponding to the six methylene groups and a multiple signal corresponding to the pyrazole proton at δ 3.42 ppm. It also showed the aromatic protons at δ 6.94–7.01 (δ, 2H, J = 8.3 Hz, Ar-H), 7.11–7.15 (δ, 2H, J = 8.2 Hz, Ar-H), 7.20–7.28 (δ, 2H, J = 8.0 Hz, Ar-H), 7.41 (s, 1H thiophene ring), 7.53–7.60 (δ, 2H, J = 8.0 Hz, Ar-H), 7.67–7.72 (δ, 2H, J = 8.1 Hz, Ar-H), 7.82–7.86 ppm (δ, 2H, J = 7.9 Hz, Ar-H), and finally two broad bands corresponding to 2 NH at d 9.15 and 10.25 ppm which were D_2O exchangeable.

Antimicrobial screening and SAR

Preliminary antimicrobial screening was carried out for all newly synthesized compounds and the results are summarized in Tables III and IV. Many of the test compounds displayed good antimicrobial activity, comparable to that of ciprofloxacin (*MIC* = 2–5 μmol mL^{-1}). Compounds **2c**, **8b**,**c**, **9b** and **10b** were the most potent ones against both Gram-negative and Gram-positive bacteria with *MIC* values ranging from 1 to 4 μmol mL^{-1}. Antibacterial activity of these compounds may result from the basic skeleton of the molecules. In addition, the presence of substituent in the phenyl ring attached to thieno[3,2-*d*]pyrimidine such as *N*-pyrimidinyl benzenesulfonamide in **2c**, 4-chloro and 4-fluorophenylpyrazolyl in **8b**,**c**, 4-chlorophenyl isoxazole in **9b** and 4-chlorophenyl thioxopyrimidinyl in **10b** should be taken into account. Compounds **2e**, **9c** and **10c** were found to be more potent against Gram-negative bacteria with *MIC* of 2–4 μmol mL^{-1} and equipotent against Gram-positive bacteria compared to ciprofloxacin. Their activity is attributed to the presence of methyl oxazole benzenesulfonamide in **2e**, 4-fluorophenyl isoxazole in **9c** and 4-fluorophenyl thioxopyrimidinyl in **10c**, which are attached to the phenyl ring of the basic skeleton.

On the other hand, compound **5b** showed slightly higher to comparable antimicrobial activity to that of the standard drug against Gram-positive bacteria and equipotent against Gram-negative bacteria, while compound **5c** showed slightly higher activity against Gram-negative bacteria, but was equipotent against Gram-positive bacteria. These activities may be due to the presence of 4-chlorophenyl-pyridinyl in **5b** and 4-fluorophenyl-pyridinyl in **5c.** Also, compounds **5a**,**d**,**e** exhibited moderate activities compared to ciprofloxacin. Compounds with methyl, like in **4a**, cyclohexyl in **4b** and phenylsulfonylthioamide in **4c**, as well as the compounds bearing amino(imino)methylsulfonyl moiety, like in **2b**, and *N*-pyridinyl-benzenesulfonamide in **2d** display good antibacterial activity.

Compounds **8e**, **9e**, **10e** exhibited good activities still lower than ciprofloxacin. These compounds are of high molecular mass and bearing a bulky substituent (thienyl group) at nitrogen in position 3 in the thieno[3,2-*d*]pyrimidine moiety. In addition, the presence of heteroatoms such as oxygen, sulfur and nitrogen plays a vital role in the observed antibacterial activity. The electronic nature of the substituent on the side-chain phenyl is an important factor in determining inhibitory potency. Thus, compounds without substituents in the phenyl group attached to the pyrazolyl in **8a,** isooxazolyl in **9a** and thioxopyrimidnyl in **10a,** or phenyl with an electron donating group [$N(CH_3)_2$] as in compounds **8d**, **9d** and **10d** exhibited moderate activities. Compounds **3**, **6a-e**, **7a-c** and **7e** displayed moderate activity, while compound **7d** exhibited moderate activity against Gram-negative bacteria but not against Gram-positive bacteria. Compound 6′-(4-chlorophenyl)-spiro[cyclohexane-1,2′-thieno[3,2-*d*][1,3]oxazin]-4′(1′*H*)-one (**1)** exhibited moderate antibacterial activity compared to ciprofloxacin.

On the other hand, compound **8c** exhibited higher antifungal activity with *MIC* of 1-2 μmol mL^{-1} than ketoconazole (*MIC* 2–3 μmol mL^{-1}), while compounds **8a**,**b** and **10b**,**c**,**e** were nearly as active as ketoconazole. Furthermore, most of the synthesized compounds, namely, **1**, **5a-d**, **6a-c**, **6e**, **7a-e**, **8d**, **9a-e**, **10a** and **10d**, exhibited moderate antifungal activity. Compounds with benzene sulfonamides showed low antifungal activity (**2a-e**, **4b,c** and **5e**), or were devoid of antifungal activity (**2d** and **4a**, **5e**).

CONCLUSIONS

Many among of the differently substituted 4-[6′-(4-chlorophenyl)-4′-oxo-1′,4′-dihydro-3′H-spiro[cyclohexane-1,2′-thieno[3,2-d]pyrimidinyl derivatives exhibit antimicrobial activity higher than the standard drug ciprofloxacin. Particularly, spirocyclohexane-1,2′-thieno[3,2-*d*]pyrimidinyl with the substituent in the phenyl ring attached to thieno[3,2-*d*] pyrimidine, such as *N*-pyrimidinyl in **2c**, *N*-(2-methyloxazolyl) benzenesulfonamide in **2e,** 4-chloro and 4-fluorophenylpyrazolyl in **8b**,**c**, 4-chlorophenyl isoxazole in **9b** and 4-chlorophenyl thioxopyrimidinyl in **10b**, showed high activity. Derivatives with 4-chlorophenyl in **5b**, 4-fluorophenyl in **5c**, methyl in **4a**, cyclohexyl in **4b** and phenyl sulfonylthioamide in **4c** showed good antibacterial activity. Compound **8c** exhibited higher antifungal activity than ketoconazole. Compounds with electron withdrawing substitutents on the phenyl ring were more potent than their analogues with electron donating substituents or with unsubstituted phenyl.

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REFERENCES

1. R. V. Chambhare, B. G. Khase, A. S. Bobde and R. H. Bahekar, Synthesis and preliminary evaluation of some N-[5-(2-furanyl)-2-methyl-4-oxo-4H-thieno[2,3-*d*]pyrimidin-3-yl]carbox-amide and 3-substituted-5-(2-furanyl)-2-methyl-3H-thieno[2,3-*d*]pyrimidin-4-ones as antimicrobial agents, *Eur. J. Med. Chem.* **38** (2003) 89–100; DOI: 10.1016/S0223-5234(02)01442-3.
2. B. V. Ashalatha, B. Narayana, K. K. V. Raj and N. S. Kumari, Synthesis of some new bioactive 3-amino-2-mercapto-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidin-4(3*H*)-one derivatives, *Eur. J. Med. Chem.* **42** (2007) 719–728; DOI: 10.1016/j.ejmech.2006.11.007.
3. Z. A. Hozien, A. A. Abdel-Wahab, K. M. Hassan, F. M. Atta and S. A. Ahmed, Synthesis of some biologically active agents derived from thieno[2,3-*d*]pyrimidine derivatives, *Pharmazie* **52** (1997) 753–758.
4. H. N. Hafez, H. A. R. Hussein and A. B. A. El-Gazzar, Design, synthesis of substituted thieno[2,3-*d*] pyrimidine-2,4-dithiones and its S-glycosides analogues as potential antiviral and antibacterial agents, *Eur. J. Med. Chem.* **45** (2010) 4026–4034; DOI: 10.1016/j.ejmech. 2010.05.060.
5. L. D. Jennings, S. L. Kincaid, Y. D. Wang, G. Krishna-Murthy, C. F. Beyer, J. P. Mc Ginnis, M. Miranda, C. M. Discafani and S. K. Rabindran, Parallel synthesis and biological evaluation of 5,6,7,8-tetrahydrobenzothieno[2,3-*d*]pyrimidin-4(3*H*)-one cytotoxic agents selective for p21-deficient cells, *Bioorg. Med. Chem. Lett.* **15** (2005) 4731–4735; DOI: 10.1016/j.bmcl.2005.07.072.

6. Y. D. Wang, S. Johnson, D. Powell, J. P. McGinnis, M. Miranda and S. K. Rabindran, Inhibition of tumor cell proliferation by thieno[2,3-*d*]pyrimidin-4(1*H*)-one-based analogs, *Bioorg. Med. Chem. Lett.* **15** (2005) 3763–3766; DOI: 10.1016/j.bmcl.2005.05.127.
7. T. Horiuchi, J. Chiba, K. Uoto and T. Soga, Discovery of novel thieno[2,3-*d*]pyrimidin-4-yl hydrazone-based inhibitors of cyclin D1-CDK4: synthesis, biological evaluation, and structure-activity relationships, *Bioorg. Med. Chem. Lett.* **19** (2009) 305–308; DOI: 10.1016/j.bmcl.2008.11.090.
8. A. Angell, C. McGuigan, L. G. Sevillano, R. Snoeck, G. Andrei, E. De Clercq and J. Balzarini, Bicyclic anti-VZV nucleosides, *Bioorg. Med. Chem. Lett.* **14** (2004) 2397–2399; DOI: 10.1016/j.bmcl.2004.03.029.
9. A. Brancale, C. McGuigan, B. Algain, P. Savy, R. Benhida, J.-L. Fourrey, G. Andrei, R. Snoeck, E. De Clercq and J. Balzarini, Bicyclic anti-VZV nucleosides: Thieno analogues retain full antiviral activity, *Bioorg. Med. Chem. Lett.* **11** (2001) 2507–2510; DOI: 10.1016/S0960-894X(01)00471-1.
10. H. N. Hafez, A. B. A. El-Gazzar and G. A. M. Nawwar, Synthesis, biological and medicinal significance of *S*-glycosido-thieno[2,3-*d*]-pyrimidines as new anti-inflammatory and analgesic agents, *Eur. J. Med. Chem.* **45** (2010) 1485–1493; DOI: 10.1016/j.ejmech.2009. 12.056.
11. A. B. A. El-Gazzar, H. A. R. Hussein and H. N. Hafez, Synthesis and biological evaluation of thieno[2,3-d]pyrimidine derivatives for anti-inflammatory, analgesic and ulcerogenic activity, *Acta Pharm.* **57** (2007) 395–411; DOI: 10.2478/v10007-007-0032-6.
12. H. N. Hafez, O. K. Al-Duaij and A. B. A. El-Gazzar, Design synthesis and pharmacological evaluation of new nonsteroidal anti-inflammatory derived from 3-aminobenzothieno[2,3-*d*] pyrimidines, *Int. J. Org. Chem.* **3** (2013) 110–118; DOI: 10.4236/ijoc.2013.32012.
13. H. N. Hafez and A. B. A. El-Gazzar, Design and synthesis of 3-pyrazolyl-thiophene, thieno[2,3-*d*] pyrimidines as new bioactive and pharmacological activity, *Bioorg. Med. Chem. Lett.* **18** (2008) 5222–5227; DOI: 10.1016/j.bmcl.2008.08.071.
14. C. J. Shishoo, V. S. Shirsath, I. S. Rathod, M. J. Patil and S. S. Bhargava, Design, synthesis and antihistaminic (H1) activity of some condensed 2-(substituted)-arylaminoethyl-pyrimidine-4-(3*H*)-ones, *Arzneimittel-Forsch.* **51** (2001) 221–231; DOI: 10.1055/s-0031-1300028.
15. M. Santagati, M. Modica, A. Santagati, F. Russo and S. Spampinato, Synthesis of amino-thienopyrimidine and thienotriazolopyrimidine derivatives as potential anti-convulsant agents, *Pharmazie* **51** (1996) 7–11.
16. A. J. Folkes, K. Ahmadi, W. K. Alderton, S. Alix, S. J. Baker, G. Box, I. S. Chuckowree, P. A. Clarke, P. Depledge, S. A. Eccles, L. S. Friedman, A. Hayes, T. C. Hancox, A. Kugendradas, L. Lensun, P. Moore, A. G. Olivero, J. Pang, S. Patel, G. H. Pergl-Wilson, F. I. Raynaud, A. Robson, N. Saghir, L. Salphati, S. Sohal, M. H. Ultsch, M. Valenti, H. J. A. Wallweber, N. C. Wan, C. Wiesmann, P. Workman, A. Zhyvoloup, M. J. Zvelebil and S. J. Shuttleworth, The identification of 2-(1*H*-indazol-4-yl)-6-(4-methanesulfonyl-piperazin-1-ylmethyl)-4-morpholin-4-yl-thieno[3,2-*d*]pyrimidine (GDC-0941) as a potent, selective, orally bioavailable inhibitor of class I PI3 kinase for the treatment of cancer, *J. Med. Chem.* **51** (2008) 5522–5532; DOI: 10.1021/jm800295d.
17. D. P. Sutherlin, L. Bao, M. Berry, G. Castanedo, I. Chuckowree, J. Dotson, A. Folks, L. Friedman, R. Goldsmith, J. Gunzner, T. Heffron, J. Lesnick, C. Lewis, S. Mathieu, J. Murray, J. Nonomiya, J. Pang, N. Pegg, W. W. Prior, L. Rouge, L. Salphati, D. Sampath, Q. Tian, V. Tsui, N. C. Wan, S. Wang, B. Q. Wei, C. Wiesmann, P. Wu, B.-Y. Zhu and A. Olivero, Discovery of a potent, selective, and orally available class I phosphatidylinositol 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) kinase inhibitor (GDC-0980) for the treatment of cancer, *J. Med. Chem.* **54** (2011) 7579–7587; DOI: 10.1021/jm2009327.
18. M. R. Prasad, R. A. Raghuram, R. P. Shanthan, R. K. Subramanian, S. Meena and K. Madhavi, Synthesis and adenosine receptor binding studies of some novel tri-azolothieno-pyrimidines, *Eur. J. Med. Chem.* **43** (2008) 614–620; DOI: 10.1016/j.ejmech.2007.05.001.
19. K. A. Chakraborti, B. Gopalakrishnan, M. E. Sobhia and M. Alpeshkumar, 3D-QSAR studies on thieno[3,2-d]pyrimidines as phosphodiesterase IV inhibitors, *Bioorg. Med. Chem. Lett.* **13** (2003) 1403–1408; DOI: 10.1016/S0960-894X(03)00172-0.

20. M. I. Crespo, L. Pages, A. Vega, V. Segarra, M. Lopez, T. Domenech, M. Miralpeix, J. Beleta, H. Ryder and J. M. Palacios, Design, synthesis and biological activities of new thieno[3,2-*d*]pyrimidines as selective type-4 phosphodiesterase inhibitors, *J. Med. Chem.* **41** (1998) 4021–4035; DOI: 10.1021/jm981012m.
21. M. Sugiyama, T. Sakamoto, K. Tabata and H. Fukumi, Condensed thienopyrimidine. IV. Synthesis and gastric antisecretory activity of 2,3-dihydro-5H-oxazolothienopyrimidine derivatives, *Chem. Pharm. Bull.* **37** (1989) 2717–2722; DOI: 10.1248/cpb.37.2717.
22. A. Gangjee, Y. Qiu, W. Li and R. L. Kisliuk, Potent dual thymidylate synthase and dihydrofolate reductase inhibitors: Classical and nonclassical 2-amino-4-oxo-5-arylthio-substituted-6-methyl-thieno[2,3-*d*]pyrimidine antifolates, *J. Med. Chem.* **51** (2008) 5789–5797; DOI: 10.1021/jm8006933.
23. N. Pemberton, E. Chorell and F. Almqvist, Microwave-assisted synthesis and functionalization of 2-pyridones, 2-quinolones and other ring-fused 2-pyridones, *Heterocycl. Chem.* **1** (2006) 1–30; DOI: 10.1007/708_006.
24. S. R. Jain and A. Kar, The antibacterial activity of some essential oils and their combination, *Planta Med.* **20** (1971) 118–123; DOI: 10.1055/s-0028-109967.
25. M. J. Weinstein and G. H. Wagman, Plant-derived antibiotics in: *Antibiotics Isolation, Separation and urification* (Ed. L. A. Mitscher), Elsevier Scientific Publishing Company, Amsterdam 1978, p. 464.
26. A. L. Barry and C. Thornsbery, Susceptibility testing, in: *Manual of Clinical Microbiology* (Ed. J. P. Lennette), American Society for Microbiology, Washington (DC) 1981, pp. 561–574.
27. K. J. Ryan and C. G. Ray (Eds.), *Sherris Medical Microbiology*, 4th ed., McGraw Hill, New York 2004.
28. K. M. Amin, M. M. Kamel, M. M. Anwar, M. Khedr and Y. M. Syam, Synthesis, biological evaluation and molecular docking of novel series of spiro [(2*H*,3*H*) quinazoline-2,10-cyclohexan]-4(1*H*)-one derivatives as anti-inflammatory and analgesic agents, *Eur. J. Med. Chem.* **45** (2010) 2117–2131; DOI**:** 10.1061/j.ejmech.2009.12.078.
29. J. Zhang, D. Ren, Y. Ma, W. Wang and H. Wu, CuO nanoparticles catalyzed simple and efficient synthesis of 2,3-dihydroquinazolin-4(1H)-ones and quinazolin-4(3*H*)-ones under ultrasound irradiation in aqueous ethanol, *Tetrahedron* **70** (2014) 5274–5282; DOI: 10.1016/j.tet.2014.05.059.