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Research Article

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Synthesis of some benzothiazole derivatives evaluated as antimicrobials and antibiofilms

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ABSTRACT

*Several new benzothiazole hybrids with other heterocyclic structures were synthesized in an attempt for exploring a new class of antibacterial, antifungal and antibiofilm agents. These derivatives include 2-(5-cyano-1,6-dihydro-6-oxo-4-arylpyrimidin-2-ylthio)-N-(6-substituted benzo [d] thiazol-2-yl)acetamide **4a-n**, 2-imino-3-(6-substituted benzo[d]thiazol-2-yl)-5-(4-(un) substituted arylidenyl)thiazolidin-4-one **6a-n** and 3-(6-Substitutedbenzo[d]thiazol-2-yl)-2-((N,N-disubstituted amino methyl)imino) thiazolidin-4-one **7a-f**. The target compounds were synthesized starting from 6-substitutedbenzo[d]thiazol-2-amine **1a, 1b** and their structures were elucidated on the basis of elemental analyses and spectral data. These compounds were screened for their antibacterial activity against gram-positive bacteria (B. subtilis, S. lutea and S. aureus), gram-negative bacteria (E. coli ATCC 25922, E. coli ATCC 5087, P. aeruginosa and P. vulgaris) and antifungal activity against C. albicans through the sensitivity test using cup plate method. Minimum inhibitory concentration was measured for the only active compounds using agar dilution method. It was shown that the two classes incorporating the 2-imino-thiazolidin-4-one structure showed more antibacterial and antifungal activities and more pronounced MIC values than the class incorporating the dihydropyrimidinone. Additionally, the antibiofilm activity of the most active compounds **6a, 6b, 6h, 6i, 6k, 6l, 7c, 7d, 7e** and **7f** as antifungals comparing to fluconazole were screened against 2 pathogenic Candida isolates CA1 and CA2 using the fluconazole as the model system. Biofilm growth was monitored semiquantitatively by colorimetric assay using the crystal violet as indicator.*

Keywords: 2-aminobenzothiazole, 6-aryl-5-cyano thiouracils, thiazolidine-4-ones, N-Mannich bases, antimicrobial, antibiofilm

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INTRODUCTION

Various diseases are due to the invasion by the pathogenic microorganisms like bacteria, fungi, virus and ricketteseas. Many potent and broad spectrum antibiotics were used to treat these infections e.g Ampicillin, Ofloxacin, Tetracycline, etc. Even though, antibiotics are life saving drugs in therapeutics, they are potentially harmful. [1,2] Moreover, biofilms represent the most prevalent type of microbial growth in nature and lead to the development of clinical infections. They can serve as a nidus for disease and are often associated with high level antimicrobial resistance of the associated organisms. Biofilm formation is an important virulence factor for a number of *Candida* species as it confers significant resistance to antifungal therapy by limiting their penetration through the matrix and protecting cells from host immune responses.[3,4] *Candida* is the fourth most common cause of blood stream infections in hospitalized patients. About 40 % of patients with *Candida* isolated from intravenous catheters having underlying fungemia and the mortality rate of patients with catheter-related candidemia approaches 40 %.[5-7] As a result of these facts, the research on new substances having high efficiency towards pathogens and less toxicity, which may be different from available resistant drugs, still of considerable interest.

Benzothiazoles are heterocyclic compounds, having various biological activities such as antimicrobial[8-17], anticancer[18], anti-inflammatory[19], anticonvulsant[20], antidiabetic[21], anti-alzheimer[22], antipsychotic[23], protein tyrosine inhibitor[24] and diuretic[25] activities. Here we synthesized two new series of benzothiazole derivatives, one incorporating the dihydropyrimidinone nucleus and the other one incorporating the 2-imino-thiazolidin-4-one structure hoping to obtain highly potent, more specific and less harmful drugs. Pyrimidines have been used as building blocks in pharmaceuticals for the synthesis of antiviral [26,27], antibacterial and antifungal [28-31] agents. Similarly, the related 5-cyano thiouracil derivatives are potential therapeutics as antiviral and antimicrobial agents.[32-34] Hybrids of benzothiazoles and pyrimidines have various biological activities such as antibacterial, antifungal, anticancer and anti-inflammatory.[35, 36] Encourage by this observation, we synthesized a new hybrid pharmacophric compounds **4a-n** in an attempt to synergize the antimicrobial potential of both hybridized groups. Such hybridization occurring between the chloroactamido derivatives of benzothiazole **2a**, **2b** [37] and 6-aryl-5-cyano-thiouracil derivatives **3a-g** moieties [38-41] in one structure happened through nucleophilic substitution reaction SN2. Thiazolidin-4-one derivatives are known to exhibit antimicrobial activity.[42-45] Also 2-imino-thiazolidin-4-ones have been found to have antifungal activity.[46-49] In the present study, we have reviewed the synthesis and different biological activities of some derivatives of 5-arylidene derivatives of benzothiazol-2-yl substituted 2- iminothiazolidin-4-ones **6a-n** and N-Mannich bases of N-substituted-2-iminothiazolidin-4-ones **7a-f**. This combination was taken in an attempt to investigate the influence of such hybridization and structural variation on the anticipated antimicrobial activity, wishing to add some synergistic biological importance to the target molecules. In continuation of our interest in the synthesis of heterocycles containing both benzothiazole and 2-imino-thiazolidin-4-one moieties, to identify new potent and less toxic candidates, we have synthesized N-Mannich bases of N-substituted-2-iminothiazolidin-4-one. The constitutions of the new products were characterized using elemental analyses, IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectral studies. For the evaluation of the antimicrobial activity, first, a disc diffusion method (Cup plate method) was used to screen the antimicrobial activity of all compounds through the determination of the inhibition of zone in mm. Then the active compounds showed by the initial sensitivity test were submitted to the second test for the determination of the minimum inhibitory concentration. Finally, only the compounds showing a good MIC against the tested fungus *Candida albicans* were subjected to the third test for the evaluation of the antibiofilm activity using two resistant strains of *Candida* forming biofilms.

EXPERIMENTAL SECTION

2.1. Chemistry:

All melting points are uncorrected and determined by the open capillary method using IA9100MK- Digital Melting Point Griffin Apparatus. Microanalyses were carried out at the microanalytical unit, Faculty of Pharmacy, Al-Azhar University. Infrared spectra were made on BRUKER Vector 22 (Japan), infrared spectrophotometers and were expressed in wavenumber (cm-1) using potassium bromide disc, at the microanalytical Center, Faculty of Science, Cairo University. The proton magnetic resonance ^{1}H-NMR and carbon magnetic resonance ^{13}C-NMR were recorded on a Bruker Avance III 400 MHz for ^{1}H and 100 MHz for ^{13}C (Bruker AG, Switzerland) with BBFO Smart Probe and Bruker 400 AEON Nitrogen-Free Magnet. Mass spectra were recorded on Fennigan MAT, SSQ 7000, Mass spectrometer, at 70 eV (EI) at the microanalytical Center, Faculty of Science, Cairo University and Waters Micromass Q-Tof Micro mass spectrometer (ESI) and Waters Acquity Ultra Performance LC with ZQ detector in ESI mode. All the compounds were named according to the IUPAC system using CS Chem. Draw Ultra version 12.0. Thin layer chromatography, using Macherey–Nagel AlugramSil G/UV254 silica gel plates and ethyl acetate-hexane as the eluting system. Compounds **2a, b**, **3a-g** and **5a, 5b** were prepared according to the reported methods [37,50, 51] while compounds **1a, b** are commercially available.

2.1.1. General method for preparation of 2-(5-Cyano-1,6- dihydro-6-oxo-4-aryl-pyrimidin-2-ylthio) N-(6-substituted benzo [d] thiazol-2-yl) acetamide, 4a-n

A mixture of 2-thiouracil derivatives **3a-g** (10 mmol) and compounds **2a**, **2b** (2.26, 2.40 gm respectively, 10 mmol) were refluxed in dry acetone (20 ml) for 12 h in presence of anhydrous potassium carbonate (1.38 gm, 10 mmol). The reaction mixture was cooled; the separated solid was filtered, washed with water, dried and crystallized from ethanol.

N-(Benzo[d]thiazol-2-yl)-2-((5-cyano-6-oxo-4-phenyl-1,6-dihydropyrimidin-2-yl)thio)acetamide (**4a**)

Yield 60%; mp: 185-188 ^{o}C. IR (cm^{-1}): 3358 (NH), 2215 (C≡N), 1653 (2C=O). ^{1}H-NMR (DMSO-d_6) δ ppm: 4.06 (s, 2H, CH_2S), 7.33 (t, 1H, C5-H, *J* = 8Hz), 7.45 (t, 1H, C6-H, *J* = 8Hz), 7.49 (t, 1H, C4'-H, *J* = 8Hz), 7.58 (s,1H, NH thiouracil exch. D_2O), 7.70 (t, 2H, C3'-H and C5'-H, *J* = 4Hz), 7.80 (d, 1H, C4-H, *J* = 8Hz), 7.97 (d, 3H, C7-H, , C2'-H and C6'-H, *J* = 8Hz), 12.34 (s, 1H, NHCO exch. D_2O.). ^{13}C-NMR (DMSO-d6) δ ppm: 34.66 (CH_2S), 85.42 (C≡N), 113.05 (C-CN), 114.34 (C4), 119.68 (C7), 121.63 (C6), 122.61 (C5), 128.94 (C2' and C6'), 132.26 (C4'), 136.62 (C3', C5'), 143.56 (C7a), 146.55 (1'), 151.53 (C4a), 153.49 (C=N thiouracil), 155.82 (C=O thiouracil),

163.51 (C=O amide), 169.15 (C-Ar), 171.49 (C2). MS m/z: 419 (M^+, 2.65 %), 420 (M^++1, 1.60 %). Anal. Calcd. For $C_{20}H_{13}N_5O_2S_2$ (419.48): C 57.26, H 3.12, and N 16.70. Found: C 57.49, H 3.17, and N 16.89.

N-(Benzo[d]thiazol-2-yl)-2-((4-(4-chlorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)acetamide (**4b**)
Yield 58%; mp: 162-165 ^oC. IR (cm^{-1}): 3427 (NH), 2210 (C≡N), 1695, 1625 (2C=O).^{1}H-NMR (DMSO-d6) δ ppm: 4.06 (s, 2H, CH_2S), 7.31 (t, 1H, C5-H, *J* = 8Hz), 7.45 (t, 1H, C6-H, *J* = 8Hz,); 7.60 (d, 2H, C2'-H and C6'-H, *J* =12Hz); 7.72 (d, 2H, C3'-H and C5'-H, *J* = 8Hz); 7.78 (d, 1H, C4-H, *J* = 8Hz); 8.00 (d, 1H, C7-H, *J* = 8Hz); 9.5 (s, 1H, NH thiouracil exch. D_2O); 12.59 (s, 1H, NHCO exch. D_2O). ^{13}C-NMR (DMSO-d6) δ ppm: 34.67 (CH_2S); 78.13 (C≡N); 118.63 (C-CN); 120.66 (C4); 122.30 (C7); 123.97 (C6); 124.27 (C5); 129.88 (C2' and C6'); 132.26 (C3' and C5'); 133.24 (C7a); 134.57 (C4'); 143.27 (C1'); 147.92 (C4a); 153.18 (C=N thiouracil); 163.80 (C=O thiouracil); 165.84 (C=O amide); 169.84 (C-Ar); 171.78(C2). MS m/z: 454 (M^+, 0.57 %), 455 (M^++1, 0.33%). Anal. Calcd. For $C_{20}H_{12}ClN_5O_2S_2$ (453.92): C 52.92, H 2.66, and N 15.43. Found: C 53.04, H 2.63, and N 15.16.

N-(Benzo[d]thiazol-2-yl)-2-((5-cyano-4-(4-nitrophenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)acetamide (**4c**)
Yield 55%; mp: 160-163 ^oC. IR (cm^{-1}): 3432 (2NH), 2214 (C≡N), 1694 (2C=O). ^{1}H-NMR (DMSO-d6) δ ppm: 4.06 (s, 2H, CH_2S); 7.32 (t, 1H, C5-H, *J* = 6Hz); 7.46 (t, 1H, C6-H, *J* = 10Hz); 7.65 (d, 2H, C2'-H and C6'-H, *J* = 12Hz); 7.75 (d, 1H, C4-H, *J* = 8Hz); 7.95 (s, 1H, NH thiouracil exch. D_2O); 7.99 (d, 1H, C7-H, *J* = 4Hz); 8.14(d, 2H, C3'-H and C5'-H, *J* = 8Hz); 12.61 (s, 1H, NHCO exch. D_2O). MS m/z: 464 (M^+, 0.54 %), 465 (M^++1, 0.34%). Anal. Calcd. For $C_{20}H_{12}N_6O_4S_2$ (464.48): C 51.72, H 2.60, and N 18.09. Found: C 51.88, H 2.62, and N 18.25.

N-(Benzo[d]thiazol-2-yl)-2-((5-cyano-4-(4-(dimethylamino)phenyl)-6-oxo-1,6-dihydropyrimidin-2-yl) thio) acetamide (**4d**)
Yield 62%; mp: 155-158 ^oC. IR (cm^{-1}): 3426 (2NH), 2206 (C≡N), 1698, 1630 (2C=O). ^{1}H-NMR (DMSO-d6) δ ppm: 3.01 (s, 6H, $N(CH_3)_2$); 3.90 (s, 2H, CH_2S); 6.90 (d, 2H, C3'-H and C5'-H, *J* = 8Hz); 7.37 (t, 1H, C5-H, *J* = 10Hz); 7.46 (t, 1H, C6-H, *J* = 8Hz); 7.57 (d, 2H, C2'-H and C6'-H, *J* = 8Hz); 7.71 (d, 1H, C4-H, *J* = 12Hz); 7.80 (s, 1H, NH thiouracil exch. D_2O); 7.99 (d, 1H, C7-H, *J* = 12Hz); 12.69 (s, 1H, NHCO exch. D_2O). MS: m/z: 462 (M^+, 0.87 %), 463 (M^++1, 0.54 %). Anal. Calcd. For $C_{22}H_{18}N_6O_2S_2$ (462.55): C 57.13, H 3.92, and N 18.17. Found: C 57.28, H 3.98, and N 18.45.

N-(Benzo[d]thiazol-2-yl)-2-((5-cyano-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)acetamide (**4e**)
Yield 65%; mp: 164-166 ^oC. IR (cm^{-1}): 3418 (2NH), 2202 (C≡N), 1720, 1683 (2C=O). 1H-NMR (DMSO-d6) δ ppm: 3.85 (s. 3H, OCH_3); 3.89 (s, 2H, CH_2S); 7.19 (d, 2H, C3'-H and C5'-H, *J* = 8Hz); 7.32 (t, 1H, C5-H, *J* = 12Hz); 7.46 (t, 1H, C6-H, *J* =10Hz); 7.69 (d, 1H, C4-H, *J* = 8Hz); 7.77 (s, 1H, NH thiouracil exch. D_2O); 7.98 (d, 2H, C2'-H and C6'-H, *J* = 12Hz); 8.10 (d,1H, C7-H, *J* = 8Hz); 8.45(s, 1H, NHCO exch. D_2O). MS; m/z: 449 (M^+, 0.87 %), 450 (M^++1, 0.48%); 451 (M^++2, 0.86%); 452 (M^++3, 0.53%). Anal. Calcd. For $C_{21}H_{15}N_5O_3S_2$ (449.51): C 56.11, H 3.36, and N 15.58. Found: C 56.34, H 3.40, and N 15.81.

N-(Benzo[d]thiazol-2-yl)-2-((4-(2-chlorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)acetamide (**4f**)
Yield 50%; mp: 148-150 ^oC. IR (cm^{-1}): 3418 (2NH), 2213 (C≡N), 1670 (2C=O). ^{1}H-NMR (DMSO-d6) δ ppm: 4.10 (s, 2H, CH_2S); 7.00 (t, 1H, C4'-H, *J* = 10Hz); 7.20 (t, 1H, C5'-H, *J* = 8Hz); 7.32 (t, 1H, C5-H, *J* = 10Hz); 7.48 (t, 1H, C6-H, *J* = 10Hz); 7.64 (s, 2H, NH thiouracil and NHCO exch. D_2O); 7.69 (d, 1H, C6'-H, *J* = 12Hz); 7.75 (d, 2H, C4-H and C3'-H, *J* =16Hz); 8.00 (d, 1H, C7-H, *J* = 12Hz). MS; m/z: 453 (M^+, 0.97 %), 454 (M^++1, 0.83%). Anal. Calcd. For $C_{20}H_{12}ClN_5O_2S_2$ (453.92): C 52.92, H 2.66, and N 15.43. Found: C 53.08, H 2.69, and N15.61.

N-(Benzo [d] thiazol-2-yl)-2-((5-cyano-4-(furan-2-yl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)acetamide (**4g**)
Yield 69%; mp: 155-157 ^oC. IR (cm-^{1}): 3428 (2NH), 2210 (C≡N), 1728, 1663 (2C=O). ^{1}H-NMR (DMSO-d6) δ ppm: 4.08 (s, 2H, CH_2S); 6.62 (s, 1H, C4'-H); 7.26 (d, 1H, C3'-H, *J* = 4Hz); 7.30 (t, 1H, C5-H, *J* = 8Hz); 7.44 (t, 1H, C6-H, *J* = 6Hz); 7.71 (d, 1H, C5'-H, *J* = 8Hz); 7.77 (d, 1H, C4-H, *J* = 8Hz); 7.82(s, 1H, NH thiouracil exch. D_2O); 7.97 (d, 1H, C7-H, *J* = 8Hz); 12.60 (s, 1H, NHCO exch. D_2O). MS: m/z: 409 (M^+, 0.98 %), 410 (M^++1, 0.79%). Anal. Calcd. For $C_{18}H_{11}N_5O_3S_2$ (409.44): C 52.80, H 2.71, and N 17.10. Found: C 52.89, H 2.69, and N 17.29.

2-((5-Cyano-6-oxo-4-phenyl-1,6-dihydropyrimidin-2-yl)thio)-N-(6-methylbenzo[d]thiazol-2-yl)acetamide (**4h**)
Yield 80%; mp: 240-242 ^oC. IR (cm^{-1}): 3349 (2NH), 2208 (C≡N), 1700, 1656 (2C=O). ^{1}H-NMR (DMSO-d6) δ ppm: 2.37 (s, 3H, CH_3); 4.05 (s, 2H, CH_2S); 7.27 (d, 1H, C5-H, *J* = 8Hz); 7.31 (t, 1H, C4'-H, *J* = 8Hz); 7.39 (t, 2H, C3'-H and C5'-H, *J* = 6Hz); 7.44 (s, 1H, NH thiouracil exch. D_2O); 7.66 (d, 1H, C4-H, *J* = 8Hz); 7.73 (d, 2H, C2'-H and C6'-H, *J* = 8Hz); 7.76 (s, 1H, C7-H); 12.53 (s, 1H, NHCO exch. D_2O). ^{13}C-NMR (DMSO-d6) δ ppm: 21.33 (CH3); 34.67 (CH2S);89.78 (C≡N); 119.98 (C-CN); 120.36 (C4); 121.64 (C7); 127.30 (C2', C6'); 128.27 (C5); 128.65 (C4'); 129.93 (C3', C5'); 131.57 (C7a); 133.61 (C6); 137.61 (1'); 146.56 (C4a); 156.87 (C=N thiouracil); 167.12 (C=O

thiouracil); 169.44 (C=O amide); 170.44 (C-Ar); 171.79 (C2). MS: m/z: 433 (M^+,32.26 %), 434 (M^++1), 30.65%). Anal. Calcd. For $C_{21}H_{15}N_5O_2S_2$ (433.51): C 58.18, H 3.49, and N 16.16. Found: C 58.26, H 3.54, and N 16.27.

2-((4-(4-Chlorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl) thio)-N- (6- methylbenzo[d]thiazol-2-yl) acetamide (**4i**)

Yield 82%; mp: 235-238 ^oC. IR (cm^{-1}): 3483, 3363 (2NH), 2213 (C≡N), 1692 (2C=O). ^{1}H-NMR (DMSO-d6) δ ppm: 2.41 (s, 3H, CH_3); 4.01 (s, 2H, CH_2S); 7.27 (d, 1H, C5-H, *J* = 8Hz); 7.37 (d, 2H, C2'-H and C6'-H, *J* = 8Hz); 7.65 (d, 1H, C4-H, *J* = 8Hz); 7.73 (d, 2H, C3'-H and C5'-H, *J* = 8Hz); 7.75 (s, 1H, C7-H); 12.48 (s, 2H, NHCO and NH thiouracil exch. D_2O). ^{13}C-NMR (DMSO-d6) δ ppm: 21.34 (CH_3); 34.69 (CH_2S); 90.09 (C≡N); 118.02 (C-CN); 120.36 (C4); 122.30 (C7); 127.93 (C5); 128.63 (C2', C6'); 130.60 (C3' and C5'); 132.63 (C4'); 133.90 (C7a); 135.56 (C6); 137.90 (1'); 147.53 (C4a); 157.86 (C=N thiouracil); 166.14 (C=O thiouracil); 169.16 (C=O amide); 170.44 (C-Ar); 172.09 (C2).MS: m/z: 467 (M^+, 25.28 %), 468 (M^++1, 16.85%); 469 (M^++2, 16.29%); 470(M^++3, 17.70%). Anal. Calcd. For $C_{21}H_{14}ClN_5O_2S_2$ (467.95): C 53.90, H 3.02, and N 14.97. Found: C 53.98, H 3.07, and N 15.04.

2-((5-Cyano-4-(4-nitrophenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(6-methylbenzo[d]thiazol-2-yl) acetamide (**4j**)

Yield 73%; mp: 218-220 ^oC. IR (cm^{-1}): 3361 (2NH), 2210 (C≡N), 1682 (2C=O). ^{1}H-NMR (DMSO-d6) δ ppm: 2.40 (s, 3H, CH_3); 4.04 (s, 2H, CH_2S); 7.26 (d, 1H, C5-H, *J* = 8Hz); 7.36 (s, 1H, NH thiouracil exch. D_2O); 7.64 (d, 1H, C4-H, *J* = 12Hz); 7.71(d, 2H, C2'-H and C6'-H, *J* = 12Hz); 7.95 (s, 1H, C7-H); 8.16 (d, 2H, C3'-H and C5'-H, *J* = 12Hz); 12.50 (s, 1H, NHCO exch. D_2O). MS: m/z: 478 (M^+, 40.57 %), 479 (M^++1), 35.43%). Anal. Calcd. For $C_{21}H_{14}N_6O_4S_2$ (478.50): C 52.71, H 2.95, and N 17.56. Found: C 52.90, H 2.93, and N 17.74.

2-((5-Cyano-4-(4-(dimethylamino)phenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(6-methylbenzo[d]thiazol-2-yl)acetamide (**4k**)

Yield 75%; mp: 248-250 ^oC. IR (cm^{-1}): 3389 (NH), 2206 (C≡N), 1683 (2C=O). ^{1}H-NMR (DMSO-d6) δ ppm: 2.41 (s, 3H, CH_3); 2.87 (s, 6H, N $(CH_3)_2$); 3.96 (s, 2H, CH_2S); 6.53 (d, 2H, C3'-H and C5'-H, *J* = 8Hz); 7.26 (d, 1H, C5-H, *J* = 8Hz); 7.65 (d, 1H, C4-H, *J* = 8Hz); 7.72 (d, 2H, C2'-H and C6'-H , *J* = 8Hz); 7.76 (s, 1H, C7-H); 12.53 (1H, NHCO, exch. D_2O). MS: m/z: 476 (M^+, 62.28), 477 (M^++1, 57.02). Anal. Calcd. For $C_{23}H_{20}N_6O_2S_2$ (476.57): C 57.97, H 4.23, and N 17.63. Found: C 58.13, H 4.29, and N 17.80.

2-((5-Cyano-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(6-methylbenzo[d]thiazol-2-yl) acetamide (**4l**)

Yield 85%; mp: 214-217 ^oC. IR (cm^{-1}): 3403, 3247 (NH), 2215 (C≡N), 1686, 1656 (2C=O). ^{1}H-NMR (DMSO-d6) δ ppm: 2.41 (s, 2H, CH_3); 3.69 (s, 3H, OCH_3); 4.05 (s, 2H, CH_2S); 6.82 (d, 2H, C3'-H and C5'-H, *J* = 8Hz); 7.26 (d, 1H, C5-H, *J* = 8Hz); 7.35 (s, 1H, NH thiouracil exch. D_2O); 7.66 (d, 3H, C4-H, C2'-H and C6'-H, *J* = 8Hz); 7.76 (s, 1H, C7-H); 12.53 (s, 1H, NHCO exch. D_2O). ^{13}C-NMR (DMSO-d6) δ ppm: 21.04 (CH_3); 35.34 (CH_2S); 55.91 (OCH_3); 86.78 (C≡N); 114.71 (C3', C5'); 120.96 (C-CN); 121.34 (C4); 122.03 (C7); 125.62 (C5); 127.97 (C2' and C6'); 132.63 (C1'); 133.61 (C7a); 134.30 (C6); 135.28 (4a); 149.19 (C4'); 158.53 (C=N thiouracil); 160.86 (C=O thiouracil); 162.55 (C=O amide); 168.17 (C-Ar);174.79 (C2). MS m/z: 463 (M^+, 85.71%); 464 (M^++1, 61.22). Anal. Calcd. For $C_{22}H_{17}N_5O_3S_2$ (463.53): C 57.00, H 3.70, and N 15.11. Found: C 57.13, H 3.76, and N 15.27.

2-((4-(2-Chlorophenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-N-(6-methylbenzo[d]thiazol-2-yl) acetamide (**4m**)

Yield 70%; mp: 153-155 ^oC. IR (cm^{-1}): 3424 (2NH), 2214 (C≡N), 1686 (2C=O). ^{1}H-NMR (DMSO-d6) δ ppm: 2.31 (s, 3H, CH_3); 4.34 (s, 2H, CH_2S); 7.02 (d, 1H, C6'-H); 7.22 (d, 1H, C5-H, J = 8Hz); 7.32 (s, 1H, NH thiouracil exch. D_2O); 7.47 (t, 1H, C5'-H, J = 8 Hz); 7.52 (t, 1H, C4'-H, J = 8Hz); 7.62 (d, 2H, C4-H and C3'-H, J = 8Hz); 7.78 (s, 1H, C7-H); 9.47 (s, 1H, NHCO exch. D_2O). MS: m/z: 467 (M^+, 1.03%); 468 (M^++1, 0.86%). Anal. Calcd. For $C_{21}H_{14}ClN_5O_2S_2$ (467.95): C 53.90, H 3.02, and N 14.97. Found: C 54.17, H 3.05, and N 15.11.

2-((5-Cyano-4-(furan-2-yl)-6-oxo-1,6-dihydropyrimidin-2-yl) thio)-N-(6-methyl benzo [d] thiazol-2-yl) acetamide (**4n**)

Yield 75%; mp: 148-150 ^oC. IR (cm^{-1}): 3420 (2NH), 2206 (C≡N), 1670 (2C=O). ^{1}H-NMR (DMSO-d6) δ ppm: 2.34 (s, 3H, CH_3); 4.01 (s, 2H, CH_2S); 6.63 (s, 1H, C4'-H, *J* = 8Hz); 7.25 (d, 1H, C5-H, *J* = 8Hz); 7.33 (s, 1H, NH thiouracil exch. D_2O); 7.64 (d, 2H, C4-H and C3'-H, *J* = 8Hz); 7.73 (s, 1H, C7-H); 7.82 (s, 1H, C5'-H); 12.55 (s, 1H, NHCO exch. D_2O). MS: m/z: 423 (M^+, 44.35%); 424 (M^++1, 59.68%). Anal. Calcd. For $C_{19}H_{13}N_5O_3S_2$ (423.47): C 53.89, H 3.09, and N 16.54. Found: C 54.04, H 3.07, and N 16.81.

2.1.2. General method for preparation of 5-Arylidene-2-imino-3-(6-substitutedbenzo[d]thiazol-2-yl) thiazolidin-4-one, **6a-n**

Imino-3-(6-substitutedbenzo[d] thiazol-2-yl)thiazolidin-4-one **5a**, **5b** (2.49 gm or 2.63 gm respectively, 10 mmol) and aromatic aldehyde (20 mmol) were added to a solution of anhydrous sodium acetate (1.64 gm, 20 mmol) in glacial acetic acid (30 ml). The mixture was heated at 100 °C for 8 h, cooled to room temperature and poured into ice water. The solid was filtered, washed with water, dried and crystallized from ethanol.

3-(Benzo[d]thiazol-2-yl)-5-benzylidene-2-iminothiazolidin-4-one (**6a**)

Yield 75%; mp: 185-188 ^{o}C. IR (cm^{-1}): 3429 (NH), 1727 (C=O), 1571 (C=NH). ^{1}H-NMR (DMSO-d6) δ ppm: 7.34 (t, 1H, C5-H, *J* = 8Hz); 7.40 (t, 1H, C6-H, *J* = 10Hz); 7.45 (t, 1H, C4'-H, *J* = 16Hz); 7.51 (t, 2H, C3'-H and C5'-H, *J* = 4Hz); 7.57 (d, 1H, C4-H, *J* = 8Hz); 7.79 (s, 1H, =CH-); 7.93(d, 1H, C7-H, *J* = 8Hz); 8.00 (d, 2H, C2'-H and C6'-H, J = 8Hz); 11.65 (s, 1H, NH exch. D_2O). MS: m/z 337 (M^+, 21.95%); 338(M^++1, 5.06%); 339(M^++2, 2.76). Anal. Calcd. For $C_{17}H_{11}N_3OS_2$ (337.42): C 60.51, H 3.29, and N 12.45. Found: C 60.69, H 3.33, and N 12.51.

3-(Benzo[d]thiazol-2-yl)-5-(4-chlorobenzylidene)-2-iminothiazolidin-4-one (**6b**)

Yield 78%; mp: 212-214 ^{o}C. IR (cm^{-1}): 3440 (NH), 1729 (C=O), 1569 (C=NH). ^{1}H-NMR (DMSO-d6) δ ppm: 7.36 (t, 1H, C5-H, *J* = 8Hz); 7.49(t, 1H, C6-H, *J* = 8Hz); 7.65 (d, 2H, C2'-H and C6'-H, *J* = 8Hz); 7.71 (d, 1H, C4-H, *J* = 12Hz); 7.78 (s, 1H, =CH-); 7.95 (d, 1H, C7-H, *J* = 8Hz); 8.00 (d, 2H, C3'-H and C5'-H, *J* = 8Hz); 12.07 (s, 1H, NH exch. D_2O). ^{13}C-NMR (DMSO-d6) δ ppm: 121.64 (-C=CH); 122.62 (C4); 123.97 (C7); 124.67 (C6); 126.99 (C5); 129.94 (C3', C5'); 131.95 (C2', C6'); 132.93 (C1'); 133.60 (C4'); 134.59 (C7a); 135.56 (C4a); 141.20 (=CH-Ar); 151.53 (C=NH thiazolidinone); 167.12 (C2); 168.77 (C=O). MS; m/z: 371 (M^+, 24.32%); 372 (M^++1, 6.45%); 373 (M^++2, 9.18%); 374 (M^++4), 3.77%). Anal. Calcd. For $C_{17}H_{10}ClN_3OS_2$ (371.86): C 54.91, H 2.71, and N 11.30. Found: C 55.08, H 2.69, and N 11.42.

3-(Benzo[d]thiazol-2-yl)-2-imino-5-(4-nitrobenzylidene)thiazolidin-4-one (**6c**)

Yield 77%; mp: 179-181 ^{o}C. IR (cm^{-1}): 3430 (NH), 1685 (C=O), 1590 (C=NH). ^{1}H-NMR (DMSO-d6) δ ppm: 7.38 (t, 1H, C5-H, *J* = 8Hz); 7.52 (t, 1H, C6-H, *J* = 10Hz); 7.79 (d, 1H, C4-H, *J* = 8Hz); 7.87 (s, 1H, =CH-); 7.96 (d, 1H, C7-H, *J* = 12Hz); 8.03 (d, 2H, C2'-H and C6'-H, *J* = 8Hz); 8.41 (d, 2H, C3'-H and C5'-H, *J* = 8Hz); 11.52 (s, 1H, NH exch. D_2O). MS: m/z 382 (M^+, 37.65%); 383 (M^++1, 9.97%); 384(M^++2, 4.96%); 385(M^++3, 1.36%). Anal. Calcd. For $C_{17}H_{10}N_4O_3S_2$ (382.42): C 53.39, H 2.64, and N 14.65. Found: C 53.45, H 2.65, and N 14.81.

3-(Benzo[d]thiazol-2-yl)-5-(4-(dimethylamino)benzylidene)-2-iminothiazolidin-4-one (**6d**)

Yield 70%; mp: 140-142 ^{o}C. IR (cm^{-1}): 3426 (NH), 1727 (C=O), 1595 (C=NH). ^{1}H-NMR (DMSO-d6) δ ppm: 3.04 (s, 6H, $N(CH_3)_2$); 6.86 (d, 2H, C3'-H and C5'-H, *J* =12Hz); 7.31 (t, 1H, C5-H, *J* = 8Hz); 7.43 (t, 1H, C6-H, *J* = 8Hz); 7.53 (d, 2H, C2'-H and C6'-H, *J* = 4Hz); 7.67 (d, 1H, C4-H, *J* = 8Hz); 7.70 (, 1H, NH exch. D_2O); 7.86 (s, 1H, =CH-); 7.95 (d, 1H, C7-H, *J* = 8Hz). MS: m/z 380 (M^+, 0.75%); 381 (M^++1, 0.51%). Anal. Calcd. For $C_{19}H_{16}N_4OS_2$ (380.49): C 59.98, H 4.24, and N 14.73. Found: C 60.13, H 4.31, and N 14.89.

3-(Benzo[d]thiazol-2-yl)-2-imino-5-(4-methoxybenzylidene)thiazolidin-4-one (**6e**)

Yield 72%; mp: 152-155 ^{o}C. IR (cm^{-1}): 3429 (NH), 1698 (C=O), 1586 (C=NH). ^{1}H-NMR (DMSO-d6) δ ppm: 3.82 (s, 3H, OCH_3); 7.15 (d, 2H, C3'-H and C5'-H, *J* = 8Hz); 7.36 (t, 1H, C5-H, *J* = 6Hz); 7.47 (t, 1H, C6-H, *J* = 4Hz); 7.66 (d, 1H, C4-H, *J* = 8Hz); 7.79 (s, 1H, =CH-); 7.93 (s, 1H, NH exch. D_2O); 7.96 (d, 2H, C2'-H and C6'-H, *J* = 8Hz); 8.01(d, 1H, C7-H, *J* = 8Hz). MS; m/z: 367 (M+, 22.58%); 368 (M^++1, 4.74%); 369 (M^++2, 4.33%); 370 (M^++3, 1.48%). Anal. Calcd. For $C_{17}H_{10}ClN_3OS_2$ (367.44): C 58.84, H 3.57, and N 11.44. Found: C 59.01, H 3.62, and N 11.57.

3-(Benzo[d]thiazol-2-yl)-5-(2-chlorobenzylidene)-2-iminothiazolidin-4-one (**6f**)

Yield 79%; mp: 207-209 ^{o}C. IR (cm^{-1}): 3429 (NH), 1721 (C=O), 1590 (C=NH). ^{1}H-NMR (DMSO-d6) δ ppm: 7.36 (t, 1H, C5-H, *J* = 8Hz); 7.48 (t, 1H, C6-H, *J* = 6Hz); 7.54 (t, 2H, C4'-H and C5'-H, *J* = 10Hz); 7.61(d, 1H, C6'-H, *J* = 8Hz); 7.66 (d, 1H, C3'-H, *J* = 8Hz); 7.74 (d, 1H, C4-H, *J* = 8Hz); 7.92 (s, 1H, =CH-); 8.01(d, 1H, C7-H, *J* = 8Hz); 12.17 (s,1H, NH exch. D_2O). ^{13}C-NMR (DMSO-d6) δ ppm: 122.33 (-C=CH); 122.57 (C4); 124.94 (C7); 127.04 (C6); 128.10 (C5);128.49 (C5'); 128.69 (C6'); 129.91 (C4'); 130.79 (C3'); 131.71 (C1'); 132.35 (C2'); 133.73 (C7a); 134.78 (C4a); 150.86 (=CH-Ar); 158.16 (C=NH thiazolidinone); 166.82 (C2); 168.18(C=O). MS; m/z: 371 (M^+, 14.77%); 372 (M^++1, 3.52%); 373(M^++2, 6.76%); 374(M^++3, 1.53%). Anal. Calcd. For $C_{17}H_{10}ClN_3OS_2$ (371.86): C 54.91, H 2.71 and N 11.30. Found: C 55.03, H 2.75 and N 11.41.

3-(Benzo[d]thiazol-2-yl)-5-(furan-2-ylmethylene)-2-iminothiazolidin-4-one (**6g**)

Yield 75%; mp: 155-158 ^{o}C. [51]

5-Benzylidene-2-imino-3-(6-methylbenzo[d]thiazol-2-yl)thiazolidin-4-one (**6h**)
Yield 65%; mp: 225-228 ^{o}C. IR (cm^{-1}): 3432 (NH), 1709 (C=O), 1585 (C=NH). ^{1}H-NMR (DMSO-d6) δ ppm: 2.42 (s, 3H, CH_3); 7.30 (d, 1H, C5-H, *J* = 8Hz); 7.49 (t, 1H, C4'-H, *J* = 8Hz); 7.57 (t, 2H, C3'-H and C5'-H, *J* = 6Hz); 7.69 (d, 3H, C4-H, C2'-H and C6'-H, *J* = 8Hz); 7.75 (s, 1H, C7-H); 7.77 (s, 1H, =CH-); 7.83 (s, 1H, NH exch. D_2O). ^{13}C-NMR (DMSO-d6) δ ppm: 21.34 (CH_3); 120.65 (-C=CH); 122.02 (C4); 124.65 (C7); 125.62 (C5); 126.99 (C4'); 127.96 (C2', C6'); 129.61 (C3', C5'); 130.60 (C7a); 132.26 (C6); 133.61 (C1'); 134.29 (=CH-Ar); 145.88 (C4a); 158.54 (C=NH thiazolidinone); 161.16 (C2); 167.79 (C=O). MS: m/z 351 (M^+, 49.84%); 352 (M^++1, 13.41%). Anal. Calcd. For $C_{18}H_{13}N_3OS_2$ (351.45): C 61.52, H 3.73 and N 11.96. Found: C 61.59, H 3.76 and N 12.05.

5-(4-Chlorobenzylidene)-2-imino-3-(6-methylbenzo[d]thiazol-2-yl)thiazolidin-4-one (**6i**)
Yield 73%; mp: 230-232 ^{o}C. IR (cm^{-1}): 3423 (NH), 1697 (C=O), 1586 (C=NH). ^{1}H-NMR (DMSO-d6) δ ppm: 2.42 (s, 3H, CH_3); 7.30 (d,1H, C5-H, *J* = 4Hz); 7.63 (d, 2 H, C2'-H and C6'-H, *J* = 8Hz); 7.69(d, 3H, C4-H, C3'-H and C5'-H, *J* = 8Hz); 7.73 (s, 1H, =CH-); 7.75 (s, 1H, NH exch. D_2O); 7.77 (s, 1H, C7-H). MS; m/z: 385 (M^+, 6.30%); 386 (M^++1, 1.40%). Anal. Calcd. For $C_{18}H_{12}ClN_3OS_2$ (385.89): C 56.02, H 3.13 and N 10.89. Found: C 56.17, H 3.09 and N 11.07.

2-Imino-3-(6-methylbenzo[d]thiazol-2-yl)-5-(4-nitrobenzylidene)thiazolidin-4-one (**6j**)
Yield 69%; mp: 210-213 ^{o}C. IR (cm^{-1}): 3437 (NH), 1725 (C=O), 1617 (C=NH). ^{1}H-NMR (DMSO-d6) δ ppm: 2.41 (s, 3H, CH_3); 7.30 (d, 1H, C5-H, *J* = 8Hz); 7.75 (d, 3H, C4-H, C2'-H and C6'-H, *J* = 4Hz); 7.81 (s, 1H, C7-H); 7.88 (s, 1H, =CH-); 8.34 (d, 2H, C3'-H and C5'-H, *J* = 8Hz); 11.52 (s, 1H, NH exch. D_2O). ^{13}C-NMR (DMSO-d6) δ ppm: 21.34 (CH_3); 122.02 (-C=CH); 123.59 (C4); 124.42 (C7); 124.74 (C5); 125.01(C3', C5'); 126.92 (C2', C6'); 127.68 (C6); 130.60 (C7a); 131.10 (C1'); 131.43 (=CH-Ar); 134.43 (C4'); 140.24 (C4a); 150.56 (C=NH thiazolidinone); 165.83 (C2); 192.34 (C=O). MS: m/z 396 (M^+, 26.62%); 397 (M^++1, 6.90%); 398 (M^++2, 1.20%). Anal. Calcd. For $C_{18}H_{12}N_4O_3S_2$ (396.44): C 54.53, H 3.05 and N 14.13. Found: C 54.62, H 3.09 and N 14.27.

5-(4-(Dimethylamino)benzylidene)-2-imino-3-(6-methylbenzo[d]thiazol-2-yl)thiazolidin-4-one (**6k**)
Yield 63%; mp: 172-175 ^{o}C. IR (cm^{-1}): 3428 (NH), 1711 (C=O), 1573 (C=NH). ^{1}H-NMR (DMSO-d6) δ ppm: 2.43 (s, 3H, CH3); 3.04 (s, 6H, $N(CH_3)_2$); 6.87 (d, 2H, C3'-H and C5'-H, *J* = 8Hz); 7.30 (d, 1H, C5-H, *J* = 12Hz); 7.53 (d, 3H, C4-H, C2'-H and C6'-H, *J* = 4Hz); 7.65 (s, 1H, =CH-); 7.76 (s, 1H, C7-H); 7.81 (s, 1H, NH exch. D_2O). MS: m/z 394 (M^+, 26.32%); 395 (M^++1, 9.90%); 396 (M^++2, 1.90%). Anal. Calcd. For $C_{20}H_{18}N_4OS_2$ (394.51): C 60.89, H 4.60 and N 14.20. Found: C 60.98, H 4.67 and N 14.33.

2-Imino-5-(4-methoxybenzylidene)-3-(6-methylbenzo[d]thiazol-2-yl)thiazolidin-4-one (**6l**)
Yield 65%; mp: 178-180 ^{o}C. IR (cm^{-1}): 3432 (NH), 1713 (C=O), 1583 (C=NH). ^{1}H-NMR (DMSO-d6) δ ppm: 2.43 (s, 3H, CH_3); 3.85 (s, 3H, OCH_3); 7.15 (d, 2H, C3'-H and C5'-H, *J* = 8Hz); 7.31 (d, 1H, C5-H, *J* = 4Hz); 7.66 (d, 3H, C4-H, C2'-H and C6'-H *J* = 8Hz); 7.73 (s, 1H, C7-H); 7.78 (s, 1H, =CH-); 7.83 (s, 1H, NH exch. D_2O). ^{13}C-NMR (DMSO-d6) δ ppm: 21.34 (CH_3); 55.90 (OCH_3); 115.01 (-C=CH); 122.02 (C4); 122.32 (C7); 123.66 (C5); 125.62 (C3', C5'); 127.98 (C1'); 132.63 (C2', C6'); 134.29 (C7a); 134.57 (C6); 137.61 (=CH-Ar); 140.43 (C4a); 149.20 (C=NH thiazolidinone); 153.86 (C4'); 161.17 (C2); 167.80 (C=O). MS: m/z 381 (M^+, 7.90%); 382 (M^++1, 2.00%). Anal. Calcd. For $C_{19}H_{15}N_3O_2S_2$ (381.47): C 59.82, H 3.96 and N 11.02. Found: C 59.97, H 3.99 and N 11.09.

5-(2-Chlorobenzylidene)-2-imino-3-(6-methylbenzo[d]thiazol-2-yl)thiazolidin-4-one (**6m**)
Yield 70%; mp: 200-202 ^{o}C. IR (cm^{-1}): 3423 (NH), 1713 (C=O), 1578 (C=NH). ^{1}H-NMR (DMSO-d6) δ ppm: 2.40 (s, 3H, CH_3); 7.26 (d, 1H, C5-H, *J* = 8Hz); 7.49 (t, 2H, C4'-H and C5'-H, *J* = 8Hz); 7.57 (d, 1H, C6'-H, *J* = 8Hz); 7.63 (d, 1H, C4-H, *J* = 8Hz); 7.69 (d, 1H, C3'-H, *J* = 4Hz); 7.72 (s, 1H, C7-H); 7.74 (s, 1H, =CH-); 12.09 (s, 1H, NH exch. D_2O).^{13}C-NMR (DMSO-d6) δ ppm: 21.35 (CH_3); 120.67 (C=CH); 121.39 (C4); 122.01 (C7); 126.79 (C5); 128.06 (C5'); 128.63 (C6'); 130.75 (C4'); 132.25 (C3'); 133.85 (C1'); 134.24 (C2'); 134.66 (C6); 135.06 (C7a); 148.52 (CH-Ar); 151.53 (C4a); 158.45 (C=NH thiazolidinone); 165.67 (C2); 174.46 (C=O). MS; m/z: 385 (M^+, 38.46%); 386 (M^++1, 23.08%). Anal. Calcd. For $C_{18}H_{12}ClN_3OS_2$ (385.89): C 56.02, H 3.13 and N 10.89. Found: C 56.17, H 3.11and N 11.07.

5-(Furan-2-ylmethylene)-2-imino-3-(6-methylbenzo[d]thiazol-2-yl)thiazolidin-4-one (**6n**)
Yield 71.5%; mp: 146-148 ^{o}C. [51]

2.1.3. General method for preparation of 3-(6-Substitutedbenzo[d]thiazol-2-yl)-2-((N,N-disubstituted amino methyl) imino)thiazolidin-4-one, 7a-f
To a solution of **7a** (2.49 gm, 100 mmol) in DMF, formaldehyde (0.6 gm, 200 mmol) was added under stirring. The reaction mixture was stirred at room temperature for 0.5 h to complete the reaction of formaldehyde and to yield methylol derivative of **7a**. To this, a solution of the appropriate secondary amine (200 mmol) in DMF was added

drop wise and refluxed for 2 h. The reaction mixture was poured into ice water, filtered off and washed with water. Finally, it was dried and purified by recrystallization from chloroform.

3-(Benzo[d]thiazol-2-yl)-2-((morpholinomethyl)imino)thiazolidin-4-one (**7a**)
Yield 70%; mp: 178-180 ^{o}C. IR (cm^{-1}): 1728 (C=O), 1567 (C=N), 1488 (-CH_2 bending of methylene bridge). ^{1}H-NMR (DMSO-d6) δ ppm: 2.78 (s, 4H, morpholine CH_2-N-CH_2); 3.65 (s, 4H, morpholine CH_2-O-CH_2); 3.90 (s, 2H, CH_2S); 4.92 (s, 2H, N-CH_2-N); 7.33 (t, 1H, C5-H, *J* = 8Hz); 7.46 (t, 1H, C6-H, *J* = 8Hz); 7.81(d, 1H, C4-H, *J* = 8Hz); 7.92 (d, 1H, C7-H, *J* =8Hz). ^{13}C-NMR (DMSO-d6) δ ppm: 33.59 (CH_2S); 51.31 (2 CH_2-N, morphline); 64.61 (2 CH_2-O, morpholine); 67.00 (N-CH_2-N); 120.96 (C4), 122.61 (C7), 124.41 (6), 126.21 (C5), 134.13 (C7a), 151.12 (C4a); 168.26 (C=N, thiazolidinone); 173.58 (C2); 176.76 (C=O). MS; m/z: 348 (M^+, 0.78%); 349 (M^++1, 0.76%). Anal. Calcd. For $C_{15}H_{16}N_4O_2S_2$ (348.44): C 51.70, H 4.63, and N 16.08. Found: C 51.88, H 4.69, and N 16.24.

3-(Benzo[d]thiazol-2-yl)-2-(((4-methylpiperazin-1-yl)methyl)imino)thiazolidin-4-one(**7b**)
Yield 65%; mp: 165-168 ^{o}C. IR (cm^{-1}): 1724 (C=O), 1556 (C=N), 1442 (-CH2 bending of methylene bridge). ^{1}H-NMR (DMSO-d6) δ ppm: 1.25 (s, 7H, N-CH_3, and CH_2-N-CH_2, piperazine); 2.66 (s, 4H, CH_2-N-CH_2, piperazine); 3.94(s, 2H, CH_2S); 4.66 (s, 2H, N-CH2-N); 7.32(t, 1H, C5-H, *J* = 8Hz); 7.44 (t, 1H, C6-H, *J* = 12Hz); 7.77 (d, 1H, C4-H, *J* = 12Hz); 7.86 (d, 1H, C7-H, *J* = 4Hz). MS; m/z: 361 (M^+, 0.32%); 362 (M^++1, 0.27%) Anal. Calcd. For $C_{16}H_{19}N_5OS_2$ (361.48): C 53.16, H 5.30, and N 19.37. Found: C 53.28, H 5.37, and N 19.45.

3-(Benzo[d]thiazol-2-yl)-2-(((dimethylamino)methyl)imino)thiazolidin-4-one (**7c**)
Yield 63%; mp: 145-148 ^{o}C. IR (cm^{-1}): 1723 (C=O), 1563 (C=N), 1485 (-CH_2 bending of methylene bridge). ^{1}H-NMR (DMSO-d6) δ ppm: 2.62 (s, 6H, N(CH_3)$_2$); 3.51 (s, 2H, N-CH_2-N); 3.93 (s, 2H, CH_2S); 7.35 (t, 1H, C5-H, *J* = 6Hz); 7.47 (t, 1H, C6-H, *J* = 8Hz); 7.81 (d, 1H, C4-H, *J* = 8Hz); 7.88 (d, 1H, C7-H, *J* = 8Hz). MS; m/z: 306 (M^+, 0.35%); 308 (M^++2, 0.61%); 309 (M^++3, 0.47%). Anal. Calcd. For $C_{13}H_{14}N_4OS_2$ (306.41): C 50.96, H 4.61, and N 18.29. Found: C 51.09, H 4.68, and N 18.48.

3-(6-Methylbenzo[d]thiazol-2-yl)-2-((morpholinomethyl)imino)thiazolidin-4-one (**7d**)
Yield 72%; mp: 210-212 ^{o}C. IR (cm^{-1}): 1702 (C=O), 1574 (C=N), 1456 (-CH_2 bending of methylene bridge). ^{1}H-NMR (DMSO-d6) δ ppm: 2.43 (s, 3H, CH_3); 2.73 (t, 4H, CH_2-N-CH_2 morpholine, *J* = 8 Hz); 3.64 (t, 4H, CH_2-O-CH_2 morpholine, *J* = 10 Hz); 3.89 (s, 2H, CH_2S); 4.88 (s, 2H, N-CH_2-N); 7.54 (s, 1H, C7-H); 7.74 (d, 2H, C4-H and C5-H, *J* = 8Hz). ^{13}C-NMR (DMSO-d6) δ ppm: 21.72 (CH_3, benzothiazole); 33.98 (CH_2S); 50.93 (2CH_2-N, morphline); 63.89 (2CH_2-O, morpholine); 66.54 (N-CH_2-N); 120.95(C4), 121.65 (C7), 127.97 (C5), 133.92 (C6), 134.28(C7a), 148.90(4a); 162.14 (C=N, thiazolidinone); 167.79 (C2); 173.44 (C=O). MS; m/z: 362 (M^+, 18.06%); 363 (M^++1, 16.11%). Anal. Calcd. For $C_{16}H_{18}N_4O_2S_2$ (362.47): C 53.02, H 5.01, and N 15.46. Found: C 53.17, H 5.08, and N 15.60.

3-(6-Methylbenzo[d]thiazol-2-yl)-2-(((4-methylpiperazin-1-yl)methyl)imino)thiazolidin-4-one (**7e**)
Yield 68%; mp: 179-182 ^{o}C. IR (cm^{-1}): 1721 (C=O), 1590 (C=N), 1452 (-CH_2 bending of methylene bridge). ^{1}H-NMR (DMSO-d6) δ ppm: 2.43 (s, 6H, CH_3 benzothiazole and N-CH_3); 2.62 (s, 8H, CH_2-N-CH_2, piperazine); 3.88 (s, 2H, CH_2S); 4.93 (s, 2H, N-CH_2-N); 7.56(s, 1H, C7-H); 7.73 (d,1H, C5-H, *J* = 8Hz); 7.78 (d, 1H, C4-H, *J* =8Hz).^{13}C-NMR (DMSO-d6) δ ppm: 26.38 (CH_3, benzothiazole); 34.97 (CH_2S); 48.59 (N-CH_3); 54.55 (4CH_2-N, piperazine); 61.85 (N-CH_2-N); 113.65 (C4), 120.36 (C7), 121.64 (C5), 129.63 (C6), 132.94 (C7a), 153.18 (C4a); 160.87 (C=N, thiazolidinone); 164.18 (C2); 170.81 (C=O). MS; m/z: 375 (M^+, 1.00%); 376 (M^++1, 0.83%). Anal. Calcd. For $C_{17}H_{21}N_5OS_2$ (375.51): C 54.37, H 5.64, and N 18.65. Found: C 54.53, H 5.73, and N 18.91.

2-(((Dimethylamino)methyl)imino)-3-(6-methylbenzo[d]thiazol-2-yl)thiazolidin-4-one (**7f**)
Yield 65%; mp: 172-174 ^{o}C. IR (cm^{-1}): 1667 (C=O), 1617 (C=N), 1506 (-CH_2 bending of methylene bridge). ^{1}H-NMR (DMSO-d6) δ ppm: 2.43 (s, 3H, CH_3); 2.64 (s, 6H, N(CH_3)$_2$); 3.94 (s, 2H, CH_2S); 4.71 (s, 2H, N-CH_2-N); 7.56 (s, 1H, C7-H); 7.73 (d, 1H, C5-H, *J* = 12Hz); 7.78 (d, 1H, C4-H, *J* = 8Hz) MS; m/z: 320 (M^+, 0.25%); 323 (M^++3, 0.29%). Anal. Calcd. For $C_{14}H_{16}N_4O_S$ (320.43): C 52.48, H 5.03, and N 17.48. Found: C 52.61, H 5.11, and N 17.64.

2.2. Antimicrobial activity:
The antimicrobial activity of 32 novel compounds **4a-n** , **6a-n** and **7a-f** including the two reported compounds **6g** and **6n**, was first screened in vitro by measuring the diameter of zone of inhibition against *Bacillus subtilis*, *Sarcina lutea* and *Staphylococcus aureus* (as representative examples of gram-positive bacteria). *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 5087, *Pseudomonas auroginosa* and *Proteus voulgaris* (as representative of gram-negative bacteria) and the fungus *Candida albicans*. Second, compounds having zones of inhibition against the tested organisms were subjected to the quantitative test for measuring the minimum inhibitory concentration.

Standard antimicrobials: For comparison, Ampicillin and Cefotaximme were used as the reference antibacterial agents while Fluconazole was employed as the reference antifungal agent.

Method for initial screening: Agar plate disc diffusion technique. [52]

Procedure: Twenty milliliters of sterilized (autoclaved at 120 °C for 30 min)Muller-Hint agar were spread in a Petri dish (13 cm in diameter) and allowed to set for 30 min. Each overnight culture of the tested microorganisms were mixed with Muller Hinton agar media to give a final turbidity of 1% microorganism equivalent to 0.5 McFarland (108 CFU/ml). A sterile cotton swab was dipped in the inoculum and the surface of the Muller Hinton agar plate was inoculated by streaking the swab over the surface. The surface of the media was allowed to dry 3-5 minute at room temperature. The sterile cork borer was used to prepare cups of 10 mm diameter. Test samples and standard drugs with volumes of 60 µl (0.6 mg/ml DMSO) were introduced into cups with the help of a micropipette. A long with the test solutions and standard drugs in each Petri dish, one cup was filled with the solvent (DMSO) which acts as the negative control. All the plates were kept at room temperature for 1 hr as a period of pre incubation diffusion to minimize effects of variations in time between applications of different solutions. So, we maintain the effective diffusion of the test drug and standard. Then, the plates were incubated at 37 ± 1 °C for 24 h. The presence of inhibition zones around the cup indicated antimicrobial activity. The diameter of the zone of inhibition was measured and recorded. Then the activity was compared with the standard drugs.

Method for quantitative screening: Agar dilution method according to Clinical Laboratory Standards Institute (CLSI). [53]

Procedure: For each sample and standard, different concentrations were diluted with Muller Hinton agar to give a final concentration ranging from (200 µg/ml – 0.7 µg/ml). DMSO was used as negative control plate. All bacterial isolates were subcultured on Brain Heart Infusion agar (B.H.I.A.) and incubated at 37 °C for 24 h. Three colonies of each microorganism were suspended in 5 ml saline, and the suspension was adjusted to 0.5 McFarland standards and then diluted 10-fold with saline to give organism suspension of (1×106 to 5×106 CFU/ml). This suspension was then further diluted by putting 1 ml suspension to 9 ml saline to give a final suspension volume of 1×105 to 5×105 CFU/ml. A multiple inoculator was used to inoculate the prepared agar plates. A 100 µl (i.e. 104 CFU) of the prepared inoculums were put in the well of multi-inoculator, where each inoculation time by multi-inoculator gave about 10 µl of prepared inoculums to the plate (i.e. 103 CFU). Each experiment was performed in duplicates. All plates were incubated at 37°C for 48 hrs. Results were recorded in terms of MIC, which is the lowest concentration of antibacterial/antifungal agent causing almost complete inhibition of growth or giving no visible growth.

2.3. Antibiofilm activity:

Only, the compounds **6a**, **6b**, **6h**, **6i**, **6k**, **6l**, **7c**, **7d**, **7e**, **7f** were screened for their antibiofilm activity using Fluconazole as antifungal standard.

Induction of Candida biofilm formation in a polystyrene, flat-bottomed, 96-well microtiter plate. [54]

2 *Candida albicans* isolates CA1 and CA2 were selected. Then *Candida albicans* biofilms were formed on commercially available presterilized, polystyrene, flat-bottomed, 96-well microtiter plates (Corning Incorporated, Corning, NY). Biofilms were formed by pipetting standardized cell suspensions (100 µl of the 1×10^6 cells/ml) into selected wells of microtiter plates. The 12^{th} column of wells on the plate should remain empty because these wells will act as negative background controls during subsequent analysis and quantification. The microtiter plate was covered with its lid, sealed with parafilm, and incubated for 48 h at 37°C. After biofilm formation, the RPMI medium was aspirated. Planktonic and nonadherent cells were removed by thoroughly washing the biofilms three times with sterile phosphate buffer saline PBS (200 µl per well). Residual PBS is removed by blotting with paper towels.

Challenging of preformed candida biofilms with antifungal agents:

Two hundreds µl of each tested drug including the reference was added to the first well of microtiter plate containing fungal biofilms. One hundred µl of RPMI per well was added to wells 2-10. Two hundreds of RPMI was placed in well 11 as a positive control. One hundred µl of antifungal agent in the first well was then removed and added to the RPMI of the second well. The last step was repeated up to the tenth wells, the final volume 100 µl was discarded. The plate was covered with its lid, sealed with parafilm, and incubated for 48 h at 37°C. After antifungal challenge, biofilms were processed and washed with sterile PBS. [55]

Quantification of the formed biofilm was done as follow:

The fixed adherent biofilm layer formed in each microtiter plate well was stained with 150 µl of 1% CV (crystal violet) for 15 min at room temperature. After staining, the stain was aspirated and excess stain was rinsed off by

placing the microtiter plate at running tap water. Washing was continued until the washing was free of the stain. Then, the microtiter plate was air dried at room temperature, the dye bound to the cells was re-solubilized by adding 150 µl of 95% ethanol to each well. The plate was covered and left at room temperature for at least 30 min without shaking. Finally, 125 µl of each well was transferred to a new plate and the OD of each well stained with CV was measured at 570 nm using the microtiter plate reader.

RESULTS AND DISCUSSION

3.1. Chemistry:

The synthetic approaches adopted to obtain the target compounds **4a-n**, **6a-n** and **7a-f** were depicted in schemes 1, 2 and 3. The structures of the newly synthesized compounds were established on the basis of their elemental analyses and spectral data. 2-Chloro-N-(6-substituted benzo[d]thiazol-2-yl)acetamide **2a, 2b** were the key starting materials for the new thiouracil derivatives **4a-n**. They were synthesized in good yields upon the reaction of equimolar amounts of the 6-substitutedbenzo [d] thiazol-2-amine **1a**, **1b** and chloroacetylchloride in dry benzene in the presence of anhydrous potassium carbonate, and refluxing for 12 hours as reported.[37] For obtaining the compounds **4a-n**, 1,2,3,4 tetrahydro-4-oxo-6-aryl-2-thioxopyrimidine-5-carbonitrile, **3a-g** were first prepared by using equimolar amounts of ethylcyanoacetate, the appropriate aromatic aldehyde and thiourea in absolute ethanol and potassium carbonate.[50] Then the condensation of the thiouracil derivatives, **3a-g** with two different alkyl chlorides **2a**, **2b** in presence of potassium carbonate using the acetone as a solvent gave 2-[5-cyano-1,6-dihydro-6-oxo-4-((un)substituted-aryl)pyrimidin-2-ylthio)-N-(6-substitutedbenzo[d]thiazol-2-yl)acetamide] derivatives **4a-n**.[56,57] Concerning the mechanism of the reaction leading to the formation of new compounds **4a-n**, we can conclude that the reaction between the two starting materials **2a**, **2b** and **3a-g** was nucleophilic substitution reaction. Being the alkyl chlorides **2a**, **2b** bearing the chlorine group are primary halides and the other starting materials **3a-g** bearing the sulfide group are moderate nucleophile so the condensation between such two starting materials was SN2 rather than SN1 reaction.[58,59] The ^{1}H-NMR of compounds **4a-n** revealed a singlet signal resonating at 3.89 to 4.10 ppm assignable to SCH_2. Also, IR spectra of **4a-n** showed bands of cyano group at 2202-2215 $cm^{-1}$and ^{13}C-NMR spectra showed the presence of cyano signal at 78.13 to 90.09 ppm along with two carbonyl signals 155.82-167.12 and 162.55-169.44 ppm. In addition, the mass spectrum of **4a** and **4h** showed molecular ion peaks at m/z 419 and 454 respectively. The synthetic pathway for the synthesis of **6a-n** and **7a-f** starts with the synthesis of 2-imino-(3-substitutedbenzothiazol-yl)-thiazolidin-4-ones **5a, 5b**.[60] In this work, benzothiazole derivatives bearing 2-imino-thiazolidin-4-one framework **5a**, **5b** were obtained in 75-78% yield as yellow solids from the 2-chloroacetamido benzothiazole derivatives I**1a**, I**1b**, ammonium thiocyanate, in absolute ethanol and reflux for 6h.[51] The second scheme involves Knoevenagel condensation of the active methylene group situated at the fifth position of the 2-imino-(3-substituted benzothiazol-2-yl)-thiazolidin-4-ones **5a**, **5b** with various aromatic aldehydes to yield 5-arylidenyl-4-thiazolidenone derivatives **6a-n**. Such condensation was carried out in acetic acid containing anhydrous sodium acetate.[61,62] Sodium acetate in glacial acetic acid formed a good buffered medium for Knoevenagel reaction to complete, so sodium acetate was used as a weakly base catalyst for such condensation. The reaction gave good yields only when the aromatic part of the aldehyde was substituted with electron withdrawing groups like a nitro or chloro group or when the aromatic aldehyde was the furfural. The yield of the reaction of unsubstituted or electron donating-substituted benzaldehyde was low. ^{1}H-NMR spectra revealed the appearance of new singlet signals at 7.65-7.92 ppm attributed to the CH olefinic of compounds **6a-n**, the disappearance of the characteristic singlet peak attributed to the CH_2 of the thiazolidinone and additional aromatic protons. Moreover, the formation of new thiazolidinones **6a-n** was assisted by the appearance of the peak of = CH-Ar group at 141.20 and 131.43 ppm in contact with the increase in the aromatic carbons for compounds **6 b** and **6j** respectively. Finally, the mass spectrum of compounds **6a**, **6h** showed molecular ion peaks at m/z 337, 351 respectively that were consistent with the molecular weight of the compounds. In the third scheme, a series of N-Mannich bases of **7a-f** have been prepared by stirring compounds **5a, 5b** with formaldehyde in DMF solvent at R.T for 0.5 h. Then a solution of the appropriate secondary amines in DMF was added dropwise and refluxed for 2h. [63] In Mannich reaction, secondary amines were employed for the activation of the formaldehyde followed by the replacement of hydrogen of the acidic imino gp with the substituted amino methyl group. Here we used the paraformaldehyde in the place of the 37% formalin as the use of paraformaldehyde proved to be beneficial in terms of better yield and purity of the products.[64-66] Spectral studies of N-Mannich bases **7a-f** have shown the following characteristic features; disappearance or decreasing the intensity of the peak corresponding to the amino group as shown by IR spectra. ^{1}H-NMR spectra have shown the absence of (1H, -NH) secondary amino group of **5a**, **5b**. This suggests that the hydrogen atom of acidic imino group has reacted with formaldehyde and secondary amines to form N,N-disubstituted amino methyl Mannich bases. This can be confirmed by the appearance of the new ^{1}H-NMR signals at the range 3.51-4.93 ppm due to (2H, -CH_2) of methylene linkage formed between acidic imino group of **5a**, **5b** and secondary amines. In addition, it appeared different peaks related to the aliphatic CH_2 groups of different secondary amines. Also, methylene bridge (N-CH_2-N) was confirmed by ^{13}C-NMR through the appearance of signals at 67.00 and 66.54 ppm for **7a** and **7d** respectively corresponding to this bridge. Finally, the

mass spectrum of compound **7a** and **7d** showed molecular ion peaks at m/z 348, 362 that were consistent with the molecular weight of the compounds.

3.2. Antimicrobial screening:

All the 32 newly synthesized compounds (10 mg/ml) and the two reported final ones **6g** and **6n** were screened initially for their antimicrobial activity in vitro using agar diffusion (cup plate) method [67] against *Bacillus subtilis*, *Sarcina lutea* and *Staphylococcus aureus* as representatives of gram positive bacteria, *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 5087, *P. aeruginosa* and *P. vulgaris* as representative of gram-negative bacteria and the fungus *Candida albicans*. The standards used were Cefotaxime (CTX), Ampicillin (AMP) as antibacterial standards and Fluconazole (FLU) as antifungal standard. The antimicrobial activity of the newly synthesized compounds was reflected qualitatively as zone of growth inhibition of the tested microorganisms (measured in mm). After that, the antimicrobial efficacy were quantitatively examined by measuring minimum inhibitory concentration using agar dilution method for only the compounds having zones of inhibition.[68] Preliminary screening results showed that gram-positive bacteria were more sensitive than the gram-negative bacteria and that confirmed by MIC results. In many cases the MIC values were parallel to results obtained by measuring diameters of zones of inhibition. Where, compounds having large zones of inhibition also have low MIC values. Yet, there are many derivatives from both classes either thiouracils or thiazolidinones showed promising in vitro antimicrobial activity by having large zones of inhibition not typically conciding with their MIC values members of the chemical series. Overall, the MIC values were used to determine the antimicrobial activity quantitatively and more reliably than initial screening results. For thiouracils **4a-n**, the most active compounds against *Bacillus subtilis* were those having the phenyl group at the 4-position of thiouracil ring **4a** (26mm, 100 µg/ml) and **4h** (23mm, 100 µg/ml) where they showed half the activity to moderate one comparing to CTX or AMP respectively. Compound **4i** had similar zone of inhibition to **4a**, however it couldn't inhibit the *B. subtilis* growth up to 200 µg/ml. The activity of thiouracils against *S. lutea* was better than that against the *B. subtilis* although their inhibition zone diameters weren't as large as those against the *B. subtilis* comparing to the antibacterial references. In addition, compound **4j** having the 4-nitrophenyl group showed the largest zone of inhibition, but, it was inactive completely at 200 µg/ml against *S. lutea*. Among thiouracil derivatives, compounds **4b** (17 mm, 25 µg/ml) and **4i** (23 mm, 25 µg/ml) having the 4-chlorophenyl group were the most active drugs against the *S. lutea* bacterium. Also, compounds **4a** (21mm), **4e** (20 mm), 4**h** (25 mm) and **4n** (18 mm) displayed good MIC value (50 µg/ml) against that bacterium comparing to both antibacterial standards. The other active thiouracil derivatives against *S. lutea* showed reasonable to low activity by having MIC values ranging from 100 µg/ml such as **4g** (15 mm) and **4l** (23 mm) to 200 µg/ml such as **4c** (21 mm) respectively. It was obviously that the only four thiouracil derivatives **4a** (16 mm), **4h** (23 mm), **4i** (19 mm) and **4j** (16 mm) showing zones of inhibition against *Staphylococcus aureus*, showed MIC value of >200 µg/ml. About the gm –ve bacteria , the MIC was measured for compound **4l** (15 mm) against *E-coli* ATCC 25922 and for **4g** (13 mm) and **4n** (18 mm) against *E-coli* ATCC 5087 and were completely inactive at 200 µg/ml. Similarly the compounds showing zones of inhibition against *P. aeruginosa* and *P. vulgaris* were inactive at 200 µg/ml even the ones having the largest zones of inhibition like **4c** (18 mm) against *P. aeruginosa* and **4j** (20 mm) against *P. vulgaris*. Indifferently, the antifungal activity showed no better results by measuring the minimum inhibitory concentration, where only **4j** (29 mm, 200 µg/ml) having the 4-nitrophenyl group and **4n** (25 mm, 100 µg/ml) having the 2-furyl group and both are 6-methyl substituted benzothiazole analogs exhibited slight to good antifungal activity respectively. As for thiazolidinone derivatives **6a-n** and **7a-f**, the results obtained through measuring the MIC (µg/ml) of only the compounds showing zones of inhibition, revealed that the microbial growth of all gram-negative bacteria couldn't be inhibited even at the maximum tested concentration 200 µg/ml. On the other hand, they showed significant antibacterial activity against gm + ve bacteria and fungus *Candida* for most compounds. Additionally, some derivatives showed broad spectrum of activity involving the fungus. As for 5-arylidene-2-imino-thiazolidin-4-one derivatives **6** against the *B. subtilis* and *S. lutea* gm +ve bacteria, only **6f** (21, 22 mm) and **6n** (20, 24 mm) against *B. subtilis* and *S. lutea* respectively or **6j** (29 mm) against *B. subtilis* couldn't inhibit the bacterial growth up to 200µg/ml. Other ones displayed MIC values ranged from 12.5 to 200 µg/ml. The compounds having the MIC value of 12.5 or 25 µg/ml were more active than Cefotaxime or strongly active comparing to Ampicillin against *B. subtilis* such as **6b** (40 mm, 25 µg/ml), **6i** (32 mm, 12.5 µg/ml), **6k** (36 mm, 25 µg/ml) and **6l** (33 mm, 25 µg/ml) while they had good activity comparing to both antibacterial standards against *S. lutea* such as **6a** (37 mm, 12.5 µg/ml) , **6b** (38 mm, 25 µg/ml), **6d** (35 mm, 25 µg/ml), **6i** (31 mm, 12.5 µg/ml), **6k** (34 mm, 12.5 µg/ml) and **6l** (32 mm, 12.5 µg/ml). Moreover, compounds **6a** (36 mm), **6c** (31 mm) and **6d** (38 mm) (MIC=50 µg/ml) displayed equipotent and better activity comparing to Cefotaxime or Ampicillin respectively against *B. subtilis* while compound **6c** (28 mm) with MIC value of 50 µg/ml against the *S. lutea* was moderately active than both standards. Other remaining active compounds showed slight to moderate activity comparing to the antibacterial reference drugs by having MIC values of 100 µg/ml like **6e** (37.34 mm) and **6m** (38.36 mm) for *B. subtilis* and *S. lutea* and **6h** (33 mm) for *S. lutea* or 200 µg/ml for **6g** (26 mm) for both bacteria, **6h** (34 mm) for *B. subtilis* and **6j** (30 mm) for *S. lutea*. Comparably, from the listed results against the *Staphylococcus aureus* bacterium using both reference drugs, it could be revealed that only few compounds showed very good activity like **6i** (32 mm, 12.5 µg/ml) and **6k** (34 mm, 25 µg/ml) or good activity like **6e** (33 mm, 100

µg/ml). It was evident, although the derivative **6b** having the 4-chlorophenyl group at the 5-position of thiazolidinone ring with the unsubstituted benzothiazol analog, showed the largest zone of inhibition against the three gm +ve bacteria, it didn't achieve the lowest MIC value. However, it remained highly active against *B. subtilis* or moderately active against *S. lutea* comparing to antibacterial references. But, it loses its activity against *S. aureus* at the maximum tested concentration. For N-Mannich derivatives **7a-f**, all derivatives showed more activity than Cefotaxime or strong activity in comparison to Ampicillin for *B. subtilis* or very good activity comparing to both references for *S. lutea* and *S. aureus* being having MIC values (12.5 and 25 µg/ml). With exception, compounds **7a** (38 mm) and **7b** (32 mm) with MIC value of (100 µg/ml) for *Bacillus* or **7b** (38 mm) with MIC value of (200 µg/ml) for *Staphylococcus* species showed slight to moderate activity comparing to ampicillin and cefotaxime respectively or very poor activity comparing to both standards. Considering the antifungal activity for both thiazolidinone derivatives **6** and **7**, compounds that displayed no activity up to 200 µg/ml against *Candida albicans* were **6c** (22 mm), **6e** (24 mm), **6f** (18 mm), **6m** (22.5 mm), **7a** (27 mm) and **7b** (24 mm). While, compound **7l** (25 µg/ml) having the 4-methoxy phenyl group with the 6-methyl substituted analog, showed the most antifungal activity. The other derivatives showed MIC values (µg/ml) ranged from (50) for **6b** (27 mm) and **6i** (25 mm), (100) for **6a** (28 mm), **6h** (22.5 mm), **6k** (25 mm), **7c** (27 mm), **7d** (28 mm), **7e** (26 mm) and **7f** (28 mm) to (200) for **6d** (25 mm), **6g** (22 mm), **6j** (26 mm) and **6n** (23.5 mm) that showed very good, moderate to slight activity comparing to Fluconazole standard respectively. Obviously, from the above results, it was shown, most thiazolidinone derivatives had broad spectrum of activity with compound **6i** having the most broad spectrum of activity followed by **7f**, (**7c** = **7d** = **7e**), **6k**, **6l**, **6b**, **6a** and finally **6d**. In terms of pharmacophore and basing on the MIC results, the effect of the methyl substitution on the benzothiazole nucleus in contact with changing the type or the electronic nature of the aryl moiety on the activity of the classes **4a-n** and **6a-n** or in contact with changing the type of the secondary amine as in N-Mannich bases **7a-f** against the sensitive bacteria and fungus wasn't consistent. Where the most activity or the broad spectrum of activity were contributed for the derivatives having the electron withdrawing 4-Cl group on the benzene ring attached at the 4-position of the thiouracil ring as in **4b** and **4i** against the *S. lutea* bacterium or attached at the 5-position of the thiazolidinone ring and having the 6-methyl group on the benzothiazole ring as in compound **6i** against all gm +ve bacteria. While, the two thiouracil derivatives **4a** and **4h** having no substitution on the benzene ring showed the lonely activity against *Bacillus subtilis*. It was also observed that the p-chloro substituted compounds **4b**, **4i** or **6b**, **6i** were more potent than the ortho substituted ones **4f**, **4m** or **6f**, **6m** against *S. lutea* or three gram positive bacteria respectively. Similarly p-Cl substituted analogs were more potent than those with p-NO_2 substituted analogs **4c**, **4j** and **6c**, **6j** against the sensitive microorganisms except thiouracil derivative **4j** was the second active compound against the tested fungus after compound **4n**. Furthermore, compounds bearing nonpolar electron donating methoxy group at para position of benzene ring attached to the 5-position of thiazolidinone ring as in compound **6l** was responsible for the most activity against the fungus *Candida albicans*. Appending the furan group instead of the phenyl ring as in compounds **4g**, **6g** and **4n**, **6n** give no marked increase in the antimicrobial activity comparing to the alternative ones having the phenyl moiety as in compounds **4a**, **6a** and **4h**, **6h** against all microorganisms. With exception, the thiouracil derivative **4n** showed the most antifungal activity and also the compound **4n** was similar in the activity to the alternative one having the phenyl ring **4h** against the *S. lutea* bacterium. For N-Mannich bases, the introduction of the secondary amines on the methylene bridge of the compounds **5a**, **5b** showed nearly similar activity for all N-Mannich derivatives. From which, compounds **7a** and **7b** having the morpholino or N-methyl piperazino groups respectively without the substitution on the benzothiazole ring displayed less activity than that ones having instead the methyl substitution on the benzothiazole ring like **7d** and **7e**. Moreover, compound **7f** having the dimethylamino fraction showed the most broad spectrum of activity. This indicated that the activity of the new synthesized N-Mannich bases may be depended on the methyl substitution rather than the type of the secondary amine. However,compound **7c** having the dimethylamino fraction on the un substituted benzothiazole analog displayed similar activity to those having the methyl substitution with the morpholino or N-methyl piperazino fraction like **7d** and **7e** respectively. This similar activity may be related to the presence of non polar electron donating dimethylamino group that can modify the absence of the 6-methyl substitution on the benzothiazole ring.

3.3. Antibiofilm activity:

Only, ten compounds that showed MIC range from 25-100 µg / ml were screened for their antibiofilm activity. A semiquantitative assay was used to measure the ability of tested thiazolidinone derivatives **6a**, **6b**, **6h**, **6i**, **6k**, **6l** , **7c**, **7d**, **7e**, **7f** and the standard Fluconazole to prevent biofilm formation of two isolates of *Candida* CA 1 and CA 2 by measuring the optical density using the crystal violet as indicator. We did application to the tested compounds and the standard Fluconazole using the sub MIC using 50 µl of each compound and the standard. We used the normal biofilms produced by the *Candida* isolates as negative controls. Depending on the values of optical density (Absorbance) measured at wave length of 570 nm through micro titer plate reader , it was shown that as the reading increases , the influence of the tested drug on inhibition the formation of the mature biofilms decreases. Therefore, more biofilm cells will absorb more light leading to high optical density values. Here, it was noticed a marked

decrease in the complexity and cellular density of the formed biofilm for each isolate compared with the normal biofilm by all tested compounds. However, no one of the ten tested compounds showed antibiofilm activity more than Flucobnazole standard. Overall, the maximum and most antibiofilm activity was noticed for compound,**6l** ($OD_{570\ nm}$ = 0.297, 0.218) having the 4-methoxy phenyl group against CA1and CA2 pathogens. The *N*-Mannich base **7f (**$OD_{570\ nm}$ = 0.266) having the dimethylamino fraction showed almost similar OD reading to the compound **6l** (MIC = 25 µg/mL) against the CA2 pathogen, although its MIC value was 100 µg/ml. On the other hand, compounds **6a** ($OD_{570\ nm}$ = 1.061, 0.862)having the un substituted phenyl group and benzothiazole moiety, **6h** ($OD_{570\ nm}$ =0.859, 0.779**)** having the un substituted phenyl group and **6k (**$OD_{570\ nm}$ =1.269, 1.205), having the 4-dimethylamino phenyl group at the 5-position of thiazolidinone and both are 6-methyl substituted benzothiazole analogs showed weak drop in the finally formed biofilm of both *Candida albicans* isolates respectively comparing to Fluconazole standard and negative controls. Other remaining compounds such as **7f** against CA1 and **6 b**, **6i**, **7c**, **7d**, **7e** against both pathogens exhibited good to moderate antibiofilm activity comparing to Fluconazole standard.

Table 1: Zone of inhibition values (mm) of compounds 4a-n showing their antibacterial and antifungal activities

Cpd	Antibacterial activity							Antifungal activity
	Gm (+ ve)			Gm (- ve)				yeast
	B. subtilis	*S. lutea*	*S. aureus*	*E. coli* ATCC 25922	*E. coli* ATCC 5087	*P. aeruginosa*	*P. vulgaris*	*C. albicans*
4a	26	21	16	-	-	16	-	19
4b	18	17	-	-	-	16.5	-	21
4c	24.5	21	-	-	-	18	17	21
4d	16	-	-	-	-	12	16	19.5
4e	24	20	-	-	-	17.5	18	22
4f	19	-	-	-	-	16.5	18	18
4g	23	15	-	-	13	-	-	18
4h	23	25	23	-	-	17	-	18
4i	26	23	19	-	-	15	-	26.5
4j	23	26	16	-	-	16	20	29
4k	18	19	-	-	-	17	16	22
4l	21	23	-	15	-	16	19	22
4m	21	-	-	-	-	12	-	20
4n	23	18	-	-	18	18	-	25
C	-	-	-	-	-	-	-	-
AMP	22	54	31	38	44	-	-	-
CTX.	25	48	25	42	52	30	31	-
FLU	-	-	-	-	-	-	-	40

(-); no activity and no zone of inhibition; C: Control (DMSO)

Table 2: Zone of inhibition values (mm) of compounds 6a-n showing their antibacterial and antifungal activities

Cpd	Antibacterial activity							Antifungal activity
	Gm (+ ve)			Gm (- ve)				yeast
	B. subtilis	*S. lutea*	*S. aureus*	*E. coli* ATCC 25922	*E. coli* ATCC 5087	*P. aeruginosa*	*P. vulgaris*	*C. albicans*
6a	36	37	36	-	23	17	-	28
6b	40	38	42	-	24	16	-	27
6c	31	28	32	-	13	16	-	22
6d	38	35	35	20	26	16	-	25
6e	37	34	33	-	24	16	-	24
6f	21	22	14	-	-	-	-	18
6g	26	26	22	-	-	-	-	22
6h	34	33	31	-	-	-	-	22.5
6i	32	31	32	-	-	16	-	25
6j	29	30	34	28	33	18	20	26
6k	36	34	34	16	-	16	-	25
6l	33	32	32	-	-	16.5	-	24
6m	38	36	36	-	-	13	-	22.5
6n	20	24	-	-	-	15	-	23.5
C	-	-	-	-	-	-	-	-
AMP	22	54	31	38	44	-	-	-
CTX.	25	48	25	42	52	30	31	-
FLU	-	-	-	-	-	-	-	40

Table 3: Zone of inhibition values (mm) of compounds 7a-f showing their antibacterial and antifungal activities

Cpd	Antibacterial activity							Antifungal activity
	Gm (+ ve)			Gm (- ve)				yeast
	B. subtilis	*S. lutea*	*S. aureus*	*E. coli ATCC 25922*	*E. coli ATCC 5087*	*P. aeruginosa*	*P. vulgaris*	*C. albicans*
7a	38	38	40	-	-	13	-	27
7b	32	36	38	-	-	16	-	24
7c	30	34	32	-	-	16	-	27
7d	34	35	39	-	-	13	-	28
7e	35	40	40	-	-	13	-	26
7f	34	38	39	-	-	16.5	-	28
C	-	-	-	-	-	-	-	-
AMP	22	54	31	38	44	-	-	-
CTX.	25	48	25	42	52	30	31	-
FLU	-	-	-	-	-	-	-	40

Table 4: MIC results of thiouracil derivatives 4a-n

Cpd.	Minimum inhibitory concentration (µg/ml)					
	Gm (+ ve)			Gm (- ve)		yeast
	B. subtilis	*S. lutea*	*S. aureus*	*P. aeruginosa*	*P. vulgaris*	*C. albicans*
4a	100	50	>200	>200	NT	>200
4b	>200	25	NT	>200	NT	>200
4c	>200	200	NT	>200	>200	>200
4d	>200	NT	NT	>200	>200	>200
4e	>200	50	NT	>200	>200	>200
4f	>200	NT	NT	>200	>200	>200
4g	>200	100	NT	NT	NT	>200
4h	100	50	>200	>200	NT	>200
4i	>200	25	>200	>200	NT	>200
4j	>200	>200	>200	>200	>200	200
4k	>200	>200	NT	>200	>200	>200
4l	>200	100	NT	>200	>200	>200
4m	>200	NT	NT	>200	NT	>200
4n	>200	50	NT	>200	NT	100
C	>200	>200	>200	>200	>200	>200
AMP	6.25	<0.7	<0.7	NT	NT	NT
CTX.	50	<0.7	<0.7	6.25	<0.7	NT
FLU	NT	NT	NT	NT	NT	12.5

NT: not tested

Table 5: MIC results of 5-arylidene-2-imino-thiazolidin-4-one derivatives 6a-n

Cpd	Minimum inhibitory concentration (µg/ml)						
	Gm (+ ve)			Gm (- ve)			yeast
	B. subtilis	*S. lutea*	*S. aureus*	*E. coli ATCC 25922*	*E. coli ATCC 5087*	*P. aeruginosa*	*C. albicans*
6a	50	12.5	>200	NT	>200	>200	100
6b	25	25	>200	NT	>200	>200	50
6c	50	50	>200	NT	>200	>200	>200
6d	50	25	>200	>200	>200	>200	200
6e	100	100	100	NT	>200	>200	>200
6f	>200	>200	>200	NT	NT	NT	>200
6g	200	200	>200	NT	NT	NT	200
6h	200	100	>200	NT	NT	NT	100
6i	12.5	12.5	12.5	NT	NT	>200	50
6j	>200	200	>200	>200	>200	>200	200
6k	25	12.5	25	>200	NT	>200	100
6l	25	12.5	>200	NT	NT	>200	25
6m	100	100	>200	NT	NT	>200	>200
6n	>200	>200	NT	NT	NT	>200	200
C	>200	>200	>200	>200	>200	>200	>200
AMP	6.25	<0.7	<0.7	6.25	6.25	NT	NT
CTX.	50	<0.7	<0.7	<0.7	<0.7	6.25	NT
FLU	NT	NT	NT	NT	NT	NT	12.5

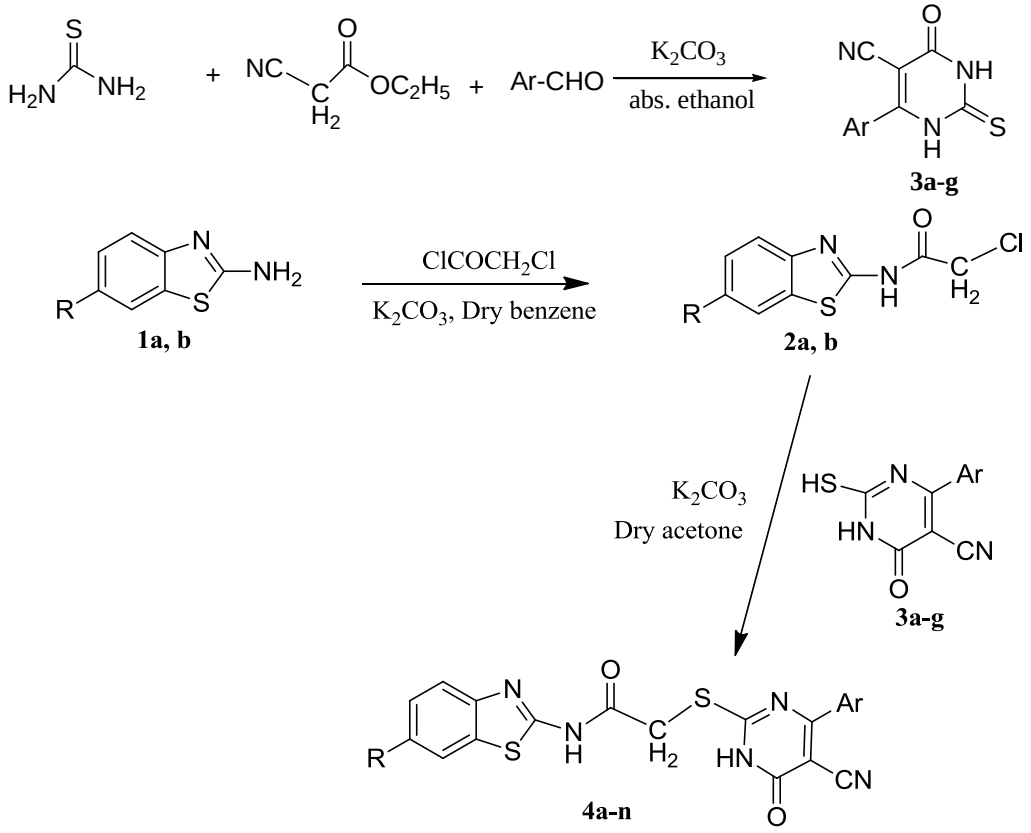
Table 6: MIC results of *N*-Mannich bases 7a-f

Cpd	Minimum inhibitory concentration (µg/ml)				
	Gm (+ ve)			Gm (- ve)	yeast
	B. subtilis	*S. lutea*	*S. aureus*	*P. aeruginosa*	*C. albicans*
7a	25	100	>200	>200	>200
7b	12.5	100	200	>200	>200
7c	12.5	12.5	25	>200	100
7d	12.5	12.5	25	>200	100
7e	12.5	12.5	25	>200	100
7f	12.5	12.5	12.5	>200	100
C	>200	>200	>200	>200	>200
AMP	6.25	<0.7	<0.7	NT	NT
CTX.	50	<0.7	<0.7	6.25	NT
FLU	NT	NT	NT	NT	12.5

Tabe 7: optical densities of antifungal thiazolidinones showing their antibiofilm activity:

Cpd	Optical density (OD_{570nm})	
	CA1 OD_{570nm} = 1.30	CA2 OD_{570nm} = 2.40
Fluconazole	0.088	0.105
6a	1.061	0.862
6b	0.307	0.74
6h	0.859	0.779
6i	0.451	0.345
6k	1.269	1.205
6l	0.297	0.218
7c	0.465	0.609
7d	0.442	0.530
7e	0.523	0.470
7f	0.521	0.266

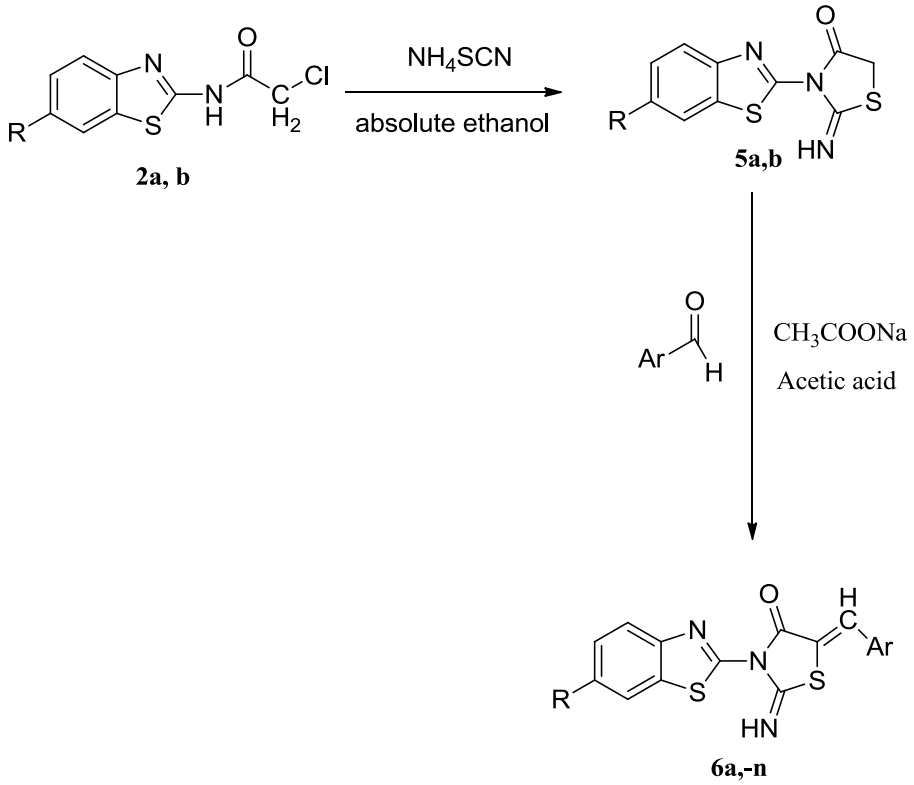
Scheme 1:



1a, **2a**, **4 a-g**, **R** = H
1b, **2 b**, **4 h-n**, **R** = CH_3

4 a, h: **Ar** = phenyl
4 b, i: **Ar** = 4-chloro phenyl
4 c, j: **Ar** = 4-nitro phenyl
4 d, k: **Ar** = 4-dimethylamino phenyl
4 e, l: **Ar** = 4-methoxy phenyl
4 f, m: **Ar** = 2-chloro phenyl
4 g, n: **Ar** = 2-furyl

Scheme 2:



2a, b

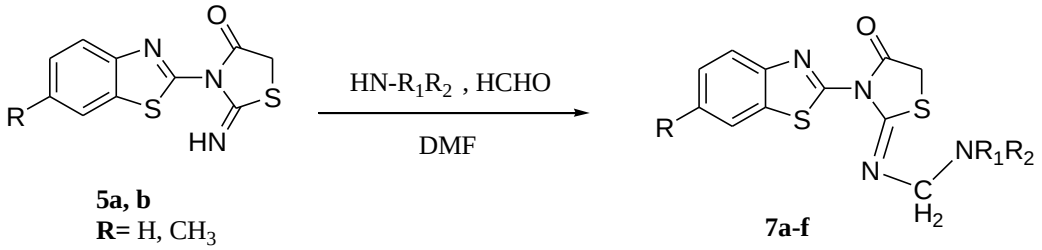
5a,b

6a,-n

2a, **5a**, **6 a-g**, **R** = H
2b, **5 b**, **6h-n**, **R** = CH_3

6 a, h: **Ar** = phenyl
6 b, i: **Ar** = 4-chloro phenyl
6 c, j: **Ar** = 4-nitro phenyl
6 d, k: **Ar** = 4-dimethylamino phenyl
6 e, l: **Ar** = 4-methoxy phenyl
6 f, m: **Ar** = 2-chloro phenyl
6 g, n: **Ar** = 2-furyl

Scheme 3:



5a, b
R= H, CH_3

7a-f

7a, d: NR_1R_2= morholino
7b, e: NR_1R_2= *N*-methyl piperazino
7c, f: NR_1R_2= *N,N*-dimethyl amino

CONCLUSION

The newly synthesized compounds **4a-n**, **6a-n** and **7a-f** presented here differed in their corresponding antimicrobial activity depending on the type of the second moiety hybridized to the benzothiazole moiety. Where, hybridization into one molecular framework between benzothiazole moiety and the 2-imino-thiazolidin-4-one moieties to give **6** and **7** gived more active compounds than such hybridization between benothiazole and 6-aryl-2-thiouracil moieties to give **4**. Based on quantitative MIC results for determining the antimicrobial activity, it was found, both hybridizations abolished the antibacterial activity against the gram-negative bacteria by showing MIC values more than 200 µg/ml. From thiouracil derivatives **4**, only compounds **4a** and **4h** having an unsubstituted phenyl moiety showed moderate to good activity comparing to Cefotaxime and Ampicillin respectively. Noticeably, thiouracil derivatives **4** showed only remarkable activity against the gram-positive bacterium *S. lutea* with the two derivatives **4b** and **4i** having the electron withdrawing Cl group at the p-position of benzene ring attached at the 4-position of thiouracil ring showed the modest activity. Moreover, the antimicrobial activity was abolished by having MIC value >200 µg/ml for thiouracil derivatives **4a**, **4h**, **4i** and **4j** that showed only zones of inhibition against *S. aureus* as gram-positive bacterium or for all thiouracil derivatives against fungus *Candida* except compounds **4j** with 4-nitro phenyl moiety and **4n** with 2-furyl moiety that showed slight to good activity respectively. Generally, hybridization between the thiazolidin-4-one nucleus and the benzothiazole nucleus displayed positive effect on the antibacterial activity against the gram positive bacteria and the antifungal activity. Compounds **6i** and **7f** showed the highest broad spectrum of activity and the most activity against all gm +ve bacteria. Also, compounds **6b**, **6i**, **6k**, **6l**, **7c**, **7d**, **7e**, **7f**, **6a**, **6c** and **6d** showed more activity than or similar one to the Cefotaxime standard against the *B.subtilis* bacterium. Considering the structure activity relationship, there is no absolute relation between the methyl substitution and either with the type, the electronic nature of the aryl moiety and the antimicrobial activity of the compounds for compounds **4**, **6** or with the type of secondary amine as in compounds **7**. However, the electron withdrawing chlorine group in compound **6i** or the non polar electron donating dimethyl amino group in compound **7f** might be responsible for increasing the spectrum of activity. Also, the substitution with electron donating 4-methoxy phenyl group as in compound **6l** might lead to the maximum antifungal activity. Moreover, the 4-chlorosubstituted analogs were more favorable than the 2-chloro or 4-nitro substituted ones for the inhibition of the gram positive bacteria or the fungus except for compound **4j** against the fungus. Finally, most of the newly synthesized thiazolidin-4-one derivatives **6** and **7** may be used for the development of new antibacterial and antifungal drugs to cure many disorders caused by the different bacterial and fungal species rather than newly synthesized thiouracil derivatives **4**. Concerning the antibiofilm activity, Most of tested antifungal agents against two pathogenic *Candida* isolates CA1 and CA2 showed significant antibiofilm activity comparing to Fluconazole reference. With the compound **6l** showed the maximum activity against the CA1 and CA2 isolates. Also, compound **7f** showed nearly similar activity to compound **6l** against CA2 pathogen. The dramatically decrease in the antibiofilm activity comparing to the standard was noticed for compounds **6a, 6h**, and **6k** with the compound **6k** was the least reactive against both pathogens. Finally, our target to synthesize compounds having antibiofilm activity achieved and on a clinical level, these results may point to approaches for preventative treatment with using the double or triple MIC concentration.

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