Full Paper

# Synthesis and Biological Evaluation of Some 2,4,5-Trisubstituted Thiazole Derivatives as Potential Antimicrobial and Anticancer Agents

**Mohammed S. Al-Saadi, Hassan M. Faidallah, and Sherif A. F. Rostom**

Department of Medicinal Chemistry, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia

We report on the synthesis and biological evaluation of two series of 2,4,5-polysubstituted thiazoles comprising the acid hydrazide functionality and some derived pharmacophores known to contribute to various chemotherapeutic activities. All newly synthesized compounds were subjected to *in-vitro* antibacterial and antifungal screening. Of the compounds tested, 13 derivatives displayed inhibitory effect on the growth of three Gram-positive strains while they lack activity against Gram-negative bacteria. Moreover, four compounds were able to exert antifungal activity against *C. albicans*. Potential antibacterial and antifungal activities were linked to the thiosemicarbazide function **6a–f** and those substituted with both the thioureido and thiosemicarbazide moieties **12a–f**. Compounds **6f** and **12f** (R = 4-F-$C_6H_4$) could be considered as the most active members in this investigation with a broad spectrum of antibacterial activity against three types of Gram-positive bacteria, together with an appreciable antifungal activity against *C. albicans*. Compounds **6d**, **6f**, and **12f** were twice as active as ampicillin against *B. subtilis*. The best antifungal activity was shown by compound **6d** 50% less active than clotrimazole. 17 compounds were selected and tested for their preliminary *in-vitro* anticancer activity according to the current one-dose protocol of the NCI. Three cell lines, non-small cell lung cancer Hop-92, ovarian cancer IGROV1, and melanoma SK-MEL-2, exhibited some sensitivity against most of the tested compounds. Compound **12f** proved to be the most active anticancer member with a broad spectrum of activity against most of the tested subpanel tumor cell lines. Consequently, **12f** was carried over to be tested in the five-dose assay.

**Keywords:** Acid hydrazides / Antibacterial / Anticancer activity / Antifungal / Thiazoles

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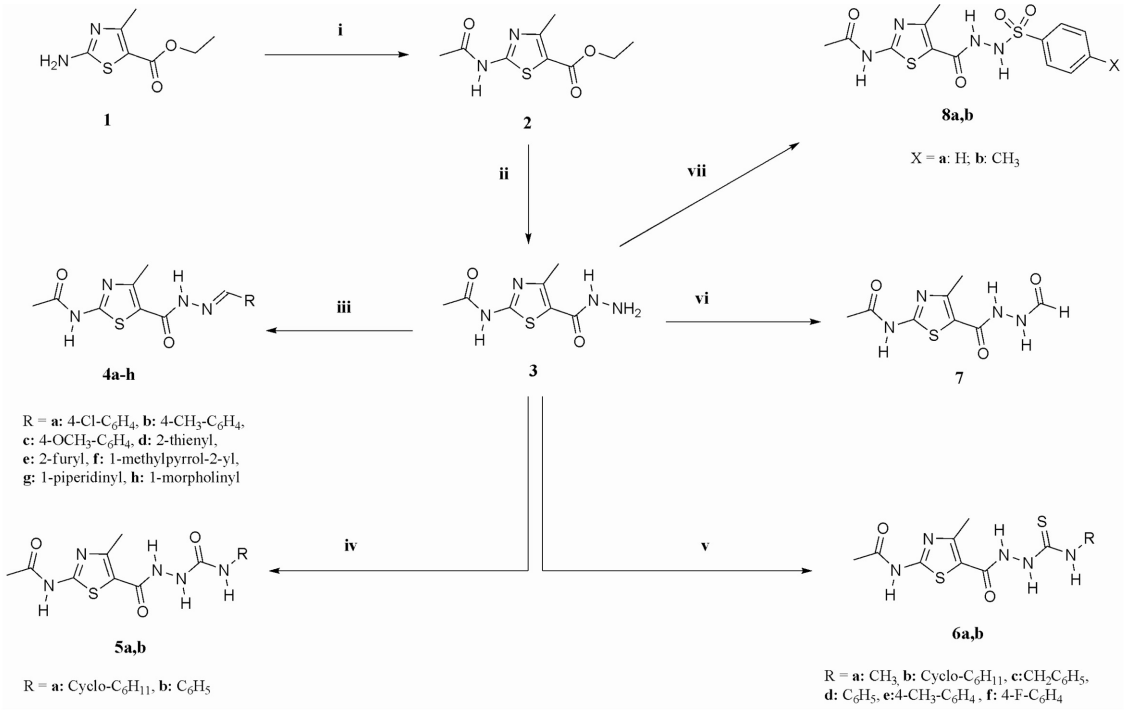
## Introduction

The past few years have witnessed an obvious reduction in the mortality caused by infectious diseases and a rise in the control of neoplastic pathologies. Nevertheless, the emergence of Gram-positive and Gram-negative pathogenic bacteria resistant to currently marketed antibacterial agents has reached an alarming level in many countries [1]. Moreover, there has been a rapid spread in primary and opportunistic fungal infections because of the increased number of immunocompromised patients (AIDS, cancer, and transplants). *Candida albicans* is one of the most common opportunistic fungi responsible for such type of infections [2]. Infections caused by these microorganisms pose a serious challenge to the medical community and highlight the importance and urgent need for new, more potent and selective non-traditional antimicrobial agents. On the other hand, chemotherapy is, at present, the only effective therapy for some types of disseminated cancers [3]. Consequently, great expecta-

**Correspondence:** Prof. Sherif A. F. Rostom, Ph.D., Associate Professor of Medicinal Chemistry, Department of Medicinal Chemistry, Faculty of Medicine, King Abdulaziz University, P.O. Box 80205, Jeddah 21589, Saudi Arabia.
**E-mail**: sherifrostom@yahoo.com
**Fax**: +966 2-6400000-22327

**Abbreviations**: inhibition zones (IZ)



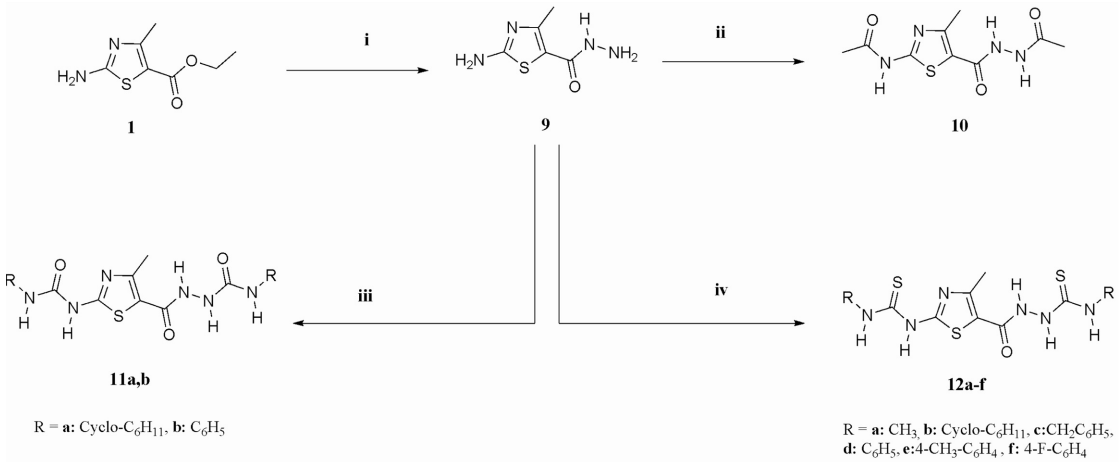

**Reagents, conditions, and yields**: *i*: $(CH_3CO)_2O$, warming, 83%; *ii*: $H_2NNH_2.H_2O$, ethanol, reflux, 70%; *iii*: Aldehyde, acetic acid, reflux, 38 – 83%; *iv*: Substituted isocyanate, pyridine, reflux, 86 – 90%; *v*: Substituted isothiocyanate, pyridine, reflux, 38 – 82%; *vi*: HCOOH, reflux, 90%; *vii*: 4-substituted benzenesulfonyl chloride, pyridine, reflux, 39 – 61%.

**Scheme 1**. Synthesis of the target compounds **2** – **8**.

tion is attached to the future development of novel potent and more selective anticancer agents. Among various heterocycles that have been explored for developing pharmaceutically important molecules, thiazoles, fused thiazoles, and thiazoles linked to various heterocylic rings through different linkages have recently attracted great attention. They were found to be associated with a wide range of chemotherapeutic activities including antimicrobial [4 – 7], antifungal [8, 9], antiparasitic [10], and antiviral [11, 12] activities. On the other hand, thiazole-containing compounds were reported to contribute to a variety of anticancer potentials including antitumor [13, 14], cytotoxic [15, 16], antiproliferative [17, 18], DNA-cleaving [19, 20], and angiogenesis inhibiting [21] activities. Interest in the chemotherapeutic activity of thiazoles was potentiated after the discovery of the natural antineoplastic antibiotics tiazofurin [22, 23], bleomycin, netropsin, and thiazole netropsin [24]. Most of these natural drugs have the thiazole carboxamide moiety as a common feature.

As a part of an ongoing research program on studying the synthesis and biological properties of some heterocyclic compounds as novel structure leads that might be of use in designing new potent and selective chemotherapeutic agents, we have reported the synthesis and *in-vitro* antimicrobial [25] and anticancer evaluation of some polysubstituted thiazole-containing compounds [26 – 30]. Some derivatives showed promising broad-spectrum antimicrobial and antitumor activities. In view of these facts, and as a continuation of our previous efforts, a new series of 2,4,5-trisubstituted thiazoles has been synthesized in the hope of discovering more active and selective antimicrobial and/or anticancer agents. The pattern of substitution of the newly designed compounds focussed on the acid hydrazide group and some derived functionalities such as the ureido, thioureido, sulfonamido, *N*-formyl, *N*-acetyl, semicarbazide, and thiosemicarbazide groups based on the reported facts about their role in exerting potential chemotherapeutic activities [13, 14, 31 ,32]. The synthesis of some Schiff' bases was not far of our interest, owing to their effective contribution as potential chemotherapeutic agents [33 – 35]. The variation in the nature and size of substituents at such functionalities was thought to be of interest representing variable electronic, lipophilic, and steric environment that would influence the targeted biological activities.




**Reagents, conditions, and yields**: *i*: $H_2NNH_2.H_2O$, ethanol, reflux, 67%; *ii*: $(CH_3CO)_2O$, warming, 73%; *iii*: Substituted isocyanate, pyridine, reflux,78–95%; *iv*: Substituted isothiocyanate, pyridine, reflux, 29-95%.

**Scheme 2**. Synthesis of the target compounds **9**–**12**.

## Results and discussion

### Chemistry

The synthetic pathways to obtain the intermediate and target compounds in this study are depicted in Schemes 1 and 2. In Scheme 1, the starting ethyl 2-amino-4-methylthiazole-5-carboxylate **1** [36] was warmed with acetic anhydride to produce the ethyl 2-acetamido-4-methylthiazole-5-carboxylate **2** in a good yield. When **2** was reacted with hydrazine hydrate, the 2-acetamido-4-methylthiazole-5-carboxylic acid hydrazide **3** was obtained, which was employed as the key intermediate in this part. Condensing **3** with the appropriate aromatic or heterocyclic aldehyde gave rise to the corresponding arylidine derivatives **4a**–**h**. Reacting the acid hydrazide **3** with different isocyanates and isothiocyanates in pyridine, afforded the corresponding 4-substituted-1-(2-acetamido-4-methylthiazole-5-carbonyl)semicarbazides **5a**, **b** and the analogous 4-substituted thiosemicarbazides **6a**–**f**, respectively. Furthermore, refluxing **3** in formic acid gave the target *N*-formyl-2-acetamido-4-methylthiazole-5-carboxylic acid hydrazide **7**. On the other hand, reacting **3** with benzenesulfonyl chloride or *p*-toluenesulfonyl chloride in the presence of pyridine led to the formation of the *N*-substituted benzenesulfonyl-2-acetamido-4-methylthiazole-5-carboxylic acid hydrazides **8a**, **b**.

At this stage, it was designed to synthesize the target carboxylic acid hydrazide **9** from the starting thiazole ester **1** and hydrazine hydrate. Acetylation of **9** with acetic anhydride furnished the *N*-acetyl-2-acetamido-4-methylthiazole-5-carboxylic acid hydrazide **10**. Finally, the synthesis of the target compounds 4-substituted-1-(2-(*N*-substituted ureido)-4-methylthiazole-5-carbonyl)semicarbazides **11a**, **b** and their isosteric 4-substituted-1-(2-(*N*-substituted thioureido)-4-methylthiazole-5-carbonyl)thiosemicarbazides **12a**–**f** could be achieved by condensing the acid hydrazide **9** with two moles of the appropriate isocyanates or isothiocyanates, respectively.

### Antimicrobial screening

All the newly synthesized compounds **2**–**12** were evaluated for their *in-vitro* antibacterial activity against *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (NRRL B-14819), and *Bacillus cereus* (ATCC 14579) as examples of Gram-positive bacteria and *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Klebsiella pneumonia* (clinical isolate) as examples of Gram-negative bacteria. They were also evaluated for their *in-vitro* antifungal potential against *Candida albicans* (ATCC 10231) and *Aspergillus niger* (recultured) fungal strains. Agar-diffusion method was used for the determination of the preliminary antibacterial and antifungal activity. Ampicillin trihydrate and clotrimazole were used as reference drugs. The results were recorded for each tested compound as the average diameter of inhibition zones (IZ) of bacterial or fungal growth around the discs in mm. The minimum inhibitory concentration (MIC) measurement was determined for compounds that showed significant growth inhibition zones (≥14 mm) using the two-fold serial dilution method [37]. The IZ (mm) and MIC (μg/mL) values are recorded in Table 1.

The results revealed that 13 out of the tested 31 compounds displayed an obvious inhibitory effect on the

**Table 1**. Inhibition zone (IZ) diameters (in mm) and minimal inhibitory concentrations (MIC, μg/mL) of the active newly synthesized compounds.

| Compound | *S. aureus* ATCC 6538 | | *B. subtilis* NRRL B-14819 | | *B. cereus* ATCC 14579 | | *C. albicans* ATCC 10231 | |
|---|---|---|---|---|---|---|---|---|
| | IZ | MIC | IZ | MIC | IZ | MIC | IZ | MIC |
| **4g** | –[a] | – | 16 | 25 | – | – | – | – |
| **5a** | 20 | 25 | – | – | – | – | – | – |
| **5b** | 17 | 50 | – | – | – | – | – | – |
| **6d** | 21 | 6.25 | 20 | 6.25 | 13 | NT[b] | 19 | 12.5 |
| **6f** | 20 | 6.25 | 20 | 6.25 | 21 | 25 | 18 | 25 |
| **9** | 14 | 100 | 10 | NT | – | – | 18 | 100 |
| **11a** | 22 | 12.5 | – | – | – | – | – | – |
| **11b** | 16 | 50 | – | – | – | – | – | – |
| **12a** | 18 | 12.5 | – | – | – | – | – | – |
| **12b** | 14 | 25 | – | – | – | – | – | – |
| **12d** | 19 | 12.5 | 16 | 25 | 9 | NT | 12 | NT |
| **12e** | 16 | 12.5 | 14 | 50 | – | – | 10 | NT |
| **12f** | 15 | 25 | 19 | 6.25 | 20 | 50 | 19 | 25 |
| **A**[c] | 36 | 6.25 | 30 | 12.5 | 32 | 12.5 | NT | NT |
| **C**[d] | – | – | – | – | – | – | 42 | 6.25 |

[a] (–): totally inactive (no inhibition zone).
[b] NT: Not tested.
[c] **A**: Ampicillin trihydrate (Standard broad spectrum antibiotic).
[d] **C**: Clotrimazole (Standard broad spectrum antifungal agent).

growth of the tested Gram-positive strains while they were totally inactive against the three tested strains of Gram-negative bacteria. Moreover, four compounds were able to exert variable degree of antifungal activity against *C. albicans*, whereas all of the tested compounds lacked antifungal activity against *Asp. niger* fungus.

As concerns the antibacterial potency of the active compounds against *S. aureus*, compounds **6d** and **6f** were equipotent to ampicillin (MIC 6.25 μg/mL), whereas the analogs **11a**, **12a**, **12d**, and **12e** (MIC 12.5 μg/mL) were 50% less active than that of ampicillin. Moreover, compounds **5a**, **12b**, and **12f** (MIC 25 μg/mL) showed 25% of the activity of ampicillin. With regard to the activity against *Bacillus subtilis*, potential activity was displayed by compounds **6d**, **6f**, and **12f** (MIC 6.25 μg/mL), which were twice as active as ampicillin (MIC 12.5 μg/mL). The analogs **4g** and **12d** (MIC 25 μg/mL) displayed half the potency of ampicillin, meanwhile, compound **12e** (MIC 50 μg/mL) showed 25% of the potency of ampicillin (MIC 12.5 μg/mL) against the same organism. *Bacillus cereus* proved to be the least sensitive Gram-positive microorganism to most of the tested compounds. Only two compounds, namely **6f** (MIC 25 μg/mL) and **12f** (MIC 50 μg/mL), exhibited remarkable growth inhibitory effect towards *B. cereus* with MIC values of 25 and 50 μg/mL, respectively (Table 1). On the other hand, investigation of the antifungal activity of the tested compounds revealed that four analogs namely **6d**, **6f**, **9**, and **12f** were able to produce appreciable growth inhibitiory activity against *C. albicans* (MIC values 12.5, 25, 25 and 100 μg/mL, respectively) comparable to that of clotrimazole (MIC 6.25 μg/mL), the standard antifungal agent utilized in this assay (Table 1).

A deep insight into the structures of the active compounds revealed that the tested compounds belong to two main structure series namely; *N*-substituted 2-acetamido-4-methylthiazole-5-carboxylic acid hydrazides **3**–**8** (Scheme 1) and the *N*-substituted 2-(substituted amino)-4-methylthiazole-5-carboxylic acid hydrazides **9**–**12** (Scheme 2).

Within the first series (Scheme 1), the nature of the substituent at the acid hydrazide functionality seems to manipulate the antimicrobial activity. Although the prototype **3** was totally inactive against all the tested microorganisms, the azomethins **4a**–**h** showed weak antimicrobial activity. Among these, compound **4g** (R = 1-piperidinyl) was the most active member, especially against the *B. subtilis*. Furthermore, derivatization of **3** into the 4-substituted semicarbazides resulted in two active compounds, namely **5a** (R = *cyclo*-$C_6H_{11}$) and **5b** (R = $C_6H_5$), active against *S. aureus* (MIC values of 25 and 50 μg/mL, respectively). On the other hand, the formation of the bioisosteric thiosemicarbazides **6a**–**f** resulted in a noticable improvement in the spectrum of antimicrobial activ-

ity. Substitution of the thiosemicarbazide group with alkyl or aralkyl moieties led to the formation of weakly active compounds **6a–c**. On the contrary, aryl substituents, as in compounds **6d–f**, showed obviously better antibacterial and antifungal activities. Therefore, compound **6d** (R = $C_6H_5$) showed high antibacterial activity against *S. aureus* and *B. subtilis* bacteria (MIC 6.25 μg/mL), in addition to a good antifungal activity against *C. albicans* (MIC 12.5 μg/mL). Substituting the phenyl moiety at position-4 with an electron-donating group (**6e**; R = 4-$CH_3$-$C_6H_4$), resulted in a dramatic reduction in activity against all the tested organisms. Whereas maximum activity within this series was obtained by introducing an electron-withdrawing group at position-4 of the phenyl moiety (**6f**; R = 4-F-$C_6H_4$) as evidenced by its MIC values, especially against *S. aureus* and *B. subtilis* bacteria (MIC 6.25 and 12.5 μg/mL, respectively).

Regarding the second series (Scheme 2), the key intermediate acid hydrazide **9** showed weak antimicrobial activity against *S. aureus, B. subtilis*, and *C. albicans.* Acetylation of **9** to the 2-acetamido-*N*-acetyl-4-methylthiazole-5-carboxylic acid hydrazide **10** led to complete abolishment of the antimicrobial activity. On the other hand, converting the parent hydrazide **9** to the 4-substituted-2-(*N*-substituted ureido)-4-methylthiazol-5-carbonyl]semicarbazide derivatives furnished two compounds **11a** (R = cyclo-$C_6H_{11}$) and **11b** (R = $C_6H_5$), active against *S. aureus* (MIC 12.5 and 50 μg/mL, respectively). Finally, bioisosteric replacement of the ureido and semicarbazide functionalities with the corresponding thioureido and thiosemicarbazide groups, resulted in a series of broad spectrum potentially active compounds **12a–f.** Unlike compounds **6a–f**, aliphatic-substituted compounds **12a** and **12b** (R = $CH_3$ and *cyclo*-$C_6H_{11}$, respectively) exhibited good activity against *S. aureus* (MIC 12.5 and 25 μg/mL, respectively). Replacing the aliphatic substituent with an aryl counterpart **12d–f**, led to an increase in the antimicrobial potential and spectrum. The 4-fluorophenyl moiety (**12f**; R = 4-F-$C_6H_4$) proved to be the most favourable substituent among this series. Compound **12f** exhibited broad spectrum antibacterial potential against *S. aureus*, *B. subtilis*, and *B. Cereus* (MIC 25, 6.25, and 50 μg/mL, respectively), in addition to a moderate antifungal activity against *C. albicans* (MIC 25 μg/mL). On the other hand, compounds **12d** (R = $C_6H_5$) and **12e** (R = 4-$CH_3$-$C_6H_4$) showed promising antibacterial activity against *S. aureus* and *B. subtilis* together with weak antifungal activity, when compared with **12f**.

### Preliminary *in-vitro* anticancer screening

Out of the newly synthesized compounds, 17 derivatives namely; **3**, **4a**, **d–h**, **5a**, **b**, **6c**, **f**, **7a**, **8a**, **10**, **11a**, **b**, **11d–h**, and **12f** were selected by the National Cancer Institute (NCI) *in-vitro* disease-oriented human cells screening panel assay to be evaluated for their *in-vitro* antitumor activity. Effective one-dose assay has been added to the NCI 60 Cell screen in order to increase compound throughput and reduce data-turnaround time to suppliers while maintaining efficient identification of active compounds [38–40]. All compounds submitted to the NCI 60 Cell screen are now tested initially at a single high dose (10, μM) in the full NCI 60 cell panel including leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer cell lines. Only compounds which satisfy pre-determined threshold inhibition criteria would progress to the five-dose screen. The threshold inhibition criteria for progression to the five-dose screen was designed to efficiently capture compounds with anti-proliferative activity and is based on careful analysis of historical Development Therapeutic Program (DTP) screening data. The data are reported as a mean graph of the percent growth of treated cells, and presented as percentage growth inhibition (GI%) caused by the test compounds (Table 2).

The obtained data revealed that some of the tested subpanel tumor cell lines exhibited pronounced sensitivity profiles against most of the tested compounds. Among these, the non-small cell lung cancer Hop-92 cell line exhibited a variable degree of sensitivity towards 14 out of the 17 compounds selected, with particular sensitivity towards compounds **3** and **12f** (percentage growth inhibition 96.2 and 80.2%, respectively). Furthermore, the growth of the melanoma SK-MEL-2 cell line was variably affected by the presence of the 17 tested compounds. Particular high activities against this cell line were shown by compounds **3**, **4d**, **4g**, **8a**, and **12f** with percentage growth inhibition values of 75.3, 80.7, 84.7, 97.1, and 96.8%, respectively. In addition, the growth of the ovarian cancer IGROV1 cell line was found to be inhibited by eleven out of the tested compounds. This cell line was clearly sensitive to compounds **4e**, **4g**, **7**, **8a**, and **10** with percentage growth inhibition values of 84.3, 90.8, 87.0, 90.7, and 76.5%, respectively. It is worth-mentioning that, only four compounds, namely; **3**, **5b**, **6c**, and **12f** were mildly able to inhibit the growth of the prostate cancer PC-3 cell line with percentage growth inhibition range of 14.8–47.9%. The rest of the tested tumor cell lines showed very weak or no sensitivity towards the tested compounds (percentage growth inhibition range 0 to $<20$%).

Further interpretation of the results revealed that compound **12f** proved to be the most active member in this study with a broad spectrum of activity against most of the tested subpanel tumor cell lines, with particular

**Table 2**. *In-vitro* percentage growth inhibition (GI%) caused by the test compounds against some selected tumor cell lines at the single-dose assay.[a)]

| Compound | NSC No. | Panel | Subpanel tumor cell lines (% growth inhibitory activity) |
|---|---|---|---|
| **3** | 745904 | Non-Small Cell Lung Cancer | HOP-92 (96.2) |
| | | Leukemia | K-562 (32.8) |
| | | Melanoma | SK-MEL-2 (75.3) |
| | | Prostate Cancer | PC-3 (15.6) |
| **4a** | 745905 | Non-Small Cell Lung Cancer | HOP-92 (71.2), NCI – H522 (57.7) |
| | | Breast Cancer | MDA-MB-468 (55.0) |
| | | Ovarian Cancer | IGROV1 (32.5) |
| | | Leukemia | PRMI-8226 (45.5) |
| | | Melanoma | SK-MEL-2 (61.4) |
| **4d** | 745906 | Non-Small Cell Lung Cancer | HOP-92 (57.4) |
| | | Breast Cancer | T-47D (32.2) |
| | | Leukemia | PRMI-8226 (38.1) |
| | | Melanoma | SK-MEL-2 (80.7) |
| **4e** | 745907 | Non-Small Cell Lung Cancer | HOP-92 (46.3) |
| | | Ovarian Cancer | IGROV1 (84.3) |
| | | Leukemia | K-562 (41.3) |
| | | Melanoma | SK-MEL-2 (65.6) |
| **4f** | 745908 | Non-Small Cell Lung Cancer | HOP-92 (43.9) |
| | | Leukemia | K-562 (45.5) |
| | | Melanoma | SK-MEL-2 (56.4) |
| **4g** | 745909 | Colon Cancer | HCC – 2998 (79.7), HCT-116 (44.2) |
| | | Ovarian Cancer | IGROV1 (90.8) |
| | | Melanoma | SK-MEL-2 (84.7) |
| **4h** | 745910 | Non-Small Cell Lung Cancer | HOP-92 (51.6), NCI – H522 (74.8) |
| | | Ovarian Cancer | IGROV1 (29.7) |
| | | Melanoma | SK-MEL-2 (65.9) |
| **5a** | 745911 | Non-Small Cell Lung Cancer | NCI – H522 (61.5) |
| | | Melanoma | SK-MEL-2 (22.6) |
| **5b** | 745912 | Non-Small Cell Lung Cancer | HOP-92 (39.4), NCI – H226 (92.8), NCI – H522 (35.5) |
| | | Breast Cancer | MDA-MB-468 (38.7) |
| | | Melanoma | SK-MEL-2 (70.1) |
| | | Prostate Cancer | PC-3 (14.8) |
| **6c** | 745913 | Non-Small Cell Lung Cancer | HOP-92 (68.5) |
| | | Breast Cancer | MDA-MB-468 (50.0) |
| | | Leukemia | CCRF-CEM (38.7), K-562 (63.8), MOLT-4 (40.7), PRMI-8226 (69.7), SR (47.9) |
| | | Renal Cancer | ACHN (42.5), UO-31 (45.4) |
| | | Melanoma | LOX IMVI (33.8), SK-MEL-2 (69.2), UACC-62 (45.8) |
| | | Prostate Cancer | PC-3 (16.3) |
| **6f** | 745914 | Non-Small Cell Lung Cancer | HOP-92 (48.6) |
| | | Ovarian Cancer | IGROV1 (27.3) |
| | | Leukemia | PRMI-8226 (64.1) |
| | | Melanoma | SK-MEL-2 (47.3), UACC-62 (39.8) |
| **7** | 745916 | Non-Small Cell Lung Cancer | HOP-92 (32.8) |
| | | Ovarian Cancer | IGROV1 (87.0) |
| | | Leukemia | K-562 (45.2) |
| | | Melanoma | SK-MEL-2 (37.3) |
| **8a** | 745917 | Colon Cancer | HCT-116 (49.7) |
| | | Ovarian Cancer | IGROV1 (90.7) |
| | | Melanoma | SK-MEL-2 (97.1) |
| **10** | 745915 | Non-Small Cell Lung Cancer | HOP-92 (42.5) |
| | | Ovarian Cancer | IGROV1 (76.5) |
| | | Leukemia | K-562 (38.6) |
| | | Melanoma | SK-MEL-2 (64.7) |
| **11a** | 745921 | Non-Small Cell Lung Cancer | HOP-92 (33.3) |
| | | Breast Cancer | MDA-MB-468 (65.5) |
| | | Ovarian Cancer | IGROV1 (29.4) |
| | | Leukemia | PRMI-8226 (45.4), SR (69.0) |
| | | Melanoma | SK-MEL-2 (17.8), M14 (32.6) |
| | | CNS Cancer | SNB-75 (38.8) |

**Table 2**. Continued

| Compound | NSC No. | Panel | Subpanel tumor cell lines (% growth inhibitory activity) |
|---|---|---|---|
| **11b** | 745922 | Non-Small Cell Lung Cancer | HOP-92 (38.3) |
| | | Ovarian Cancer | IGROV1 (39.6) |
| | | Renal Cancer | UO-31 (40.3) |
| | | Melanoma | SK-MEL-2 (47.1) |
| **12f** | 745923 | Non-Small Cell Lung Cancer | A 549 / ATCC (33.8), EKVX (36.8), HOP-92 (80.2), NCI – H226 (39.3), NCI – H23 (62.0), NCI – H522 (66.1) |
| | | Colon Cancer | HCC – 2998 (31.6), HCT-116 (55.1), HCT-15 (38.1), HT29 (38.1) |
| | | Breast Cancer | MDA-MB-468 (55.5), T-47D (64.4) |
| | | Ovarian Cancer | IGROV1 (48.3), OVCAR-3 (43.4), OVCAR-3 (57.2) |
| | | Leukemia | CCRF-CEM (60.0), HL-60 (TB) (51.7), K-562 (48.6), MOLT-4 (63.5) |
| | | Renal Cancer | A498 (46.4), ACHN (34.7), TK-10 (56.5), UO-31 (61.3) |
| | | Melanoma | M14 (49.0), SK-MEL-2 (96.8), SK-MEL-5 (51.3), UACC-62 (63.1) |
| | | Prostate Cancer | DU-145 (18.0), PC-3 (47.9) |
| | | CNS Cancer | SF-295 (59.4), U251 (47.0) |

[a] The data obtained from NCI's *in vitro* disease-oriented human tumor cell screen at 10 μM conc.

**Table 3**. Physicochemical and analytical data for compounds **2**–**12**.

| Compound | R or X | Yield (%) | M.p. (°C) (Crystallization Solvent (s))* | Molecular Formula (Mol. Weight)[a] |
|---|---|---|---|---|
| **2** | – | 83 | 220–222 (E) | $C_9H_{12}N_2O_3S$ (228.27) |
| **3** | – | 70 | 170–171 (E) | $C_7H_{10}N_4O_2S$ (214.24) |
| **4a** | 4-Cl-$C_6H_4$ | 83 | 208–209 (A/W) | $C_{14}H_{13}ClN_4O_2S$ (336.80) |
| **4b** | 4-$CH_3$-$C_6H_4$ | 65 | 156–158 (E) | $C_{15}H_{16}N_4O_2S$ (316.38) |
| **4c** | 4-$OCH_3$-$C_6H_4$ | 73 | 214–216 (E) | $C_{15}H_{16}N_4O_3S$ (332.38) |
| **4d** | 2-thienyl | 67 | 220–221 (A/W) | $C_{12}H_{12}N_4O_2S_2$ (308.38) |
| **4e** | 2-furyl | 58 | 168–169 (E) | $C_{12}H_{12}N_4O_3S$ (292.31) |
| **4f** | 1-methylpyrrol-2-yl | 43 | 160–162 (E/W) | $C_{13}H_{15}N_5O_2S$ (305.36) |
| **4g** | 1-piperidinyl | 38 | 158–160 (E/W) | $C_{13}H_{19}N_5O_2S$ (309.39) |
| **4h** | 1-morpholinyl | 50 | 154–156 (E/W) | $C_{12}H_{17}N_5O_3S$ (311.36) |
| **5a** | *cyclo*-$C_6H_{11}$ | 86 | 162–163 (E) | $C_{14}H_{21}N_5O_3S$ (339.41) |
| **5b** | $C_6H_5$ | 90 | 200–201 (E) | $C_{14}H_{15}N_5O_3S$ (333.37) |
| **6a** | $CH_3$ | 38 | 190–192 (E) | $C_9H_{13}N_5O_2S_2$ (287.36) |
| **6b** | *cyclo*-$C_6H_{11}$ | 53 | 166–168 (E) | $C_{14}H_{21}N_5O_2S_2$ (355.48) |
| **6c** | $CH_2$-$C_6H_5$ | 47 | 136–137 (M) | $C_{15}H_{17}N_5O_2S_2$ (363.46) |
| **6d** | $C_6H_5$ | 69 | 120–122 (E) | $C_{14}H_{15}N_5O_2S_2$ (349.43) |
| **6e** | 4-$CH_3$-$C_6H_4$ | 82 | 158–159 (E) | $C_{15}H_{17}N_5O_2S_2$ (363.46) |
| **6f** | 4-F-$C_6H_4$ | 71 | 148–150 (E) | $C_{14}H_{14}FN_5O_2S_2$ (367.42) |
| **7** | – | 90 | 224–226 (E/W) | $C_8H_{10}N_4O_3S$ (242.26) |
| **8a** | H | 61 | 180–182 (A) | $C_{13}H_{14}N_4O_4S_2$ (354.40) |
| **8b** | $CH_3$ | 39 | 148–149 (A) | $C_{14}H_{16}N_4O_4S_2$ (368.43) |
| **9** | – | 67 | 142–144 (M) | $C_5H_8N_4OS$ (172.21) |
| **10** | – | 73 | 172–173 (E/W) | $C_9H_{12}N_4O_3S$ (256.28) |
| **11a** | *cyclo*-$C_6H_{11}$ | 95 | 161–163 (E) | $C_{19}H_{30}N_6O_3S$ (422.54) |
| **11b** | $C_6H_5$ | 78 | 228–230 (E) | $C_{19}H_{18}N_6O_3S$ (410.45) |
| **12a** | $CH_3$ | 40 | 200–202 (E/PE) | $C_9H_{14}N_6OS_3$ (318.44) |
| **12b** | *cyclo*-$C_6H_{11}$ | 95 | 204–206 (E) | $C_{19}H_{30}N_6OS_3$ (454.68) |
| **12c** | $CH_2$-$C_6H_5$ | 29 | 142–144 (DMF/E) | $C_{21}H_{22}N_6OS_3$ (470.63) |
| **12d** | $C_6H_5$ | 85 | 138–140 (D/W) | $C_{19}H_{18}N_6OS_3$ (442.58) |
| **12e** | 4-$CH_3$-$C_6H_4$ | 65 | 156–158 (D/W) | $C_{21}H_{22}N_6OS_3$ (470.63) |
| **12f** | 4-F-$C_6H_4$ | 83 | 187–189 (DMF/W) | $C_{19}H_{16}F_2N_6OS_3$ (478.56) |

* Crystallization Solvent (s): A: Acetic acid, D: Dioxane, DMF: *N,N*-Dimethylformamide, E: Ethanol, M: Methanol, PE: Petroleum ether (60:80), W: Water.

[a] The found values (F) are within ± 0.4% of the calculated (C) values.

effectiveness against the non-small cell lung cancer Hop-92 and melanoma SK-MEL-2 cell lines (GI% 80.2 and 96.8, respectively). Interestingly, compound **12f** has satisfied the pre-determined threshold inhibition criteria and consequently was carried over to be tested in the five-dose assay.

## Conclusion

In conclusion, the results obtained in this study revealed that 13 compounds out of the newly synthesized compounds displayed a variable degree of antibacterial activity against Gram-positive bacteria, particularly *S. aureus* and *B. subtilis*. Meanwhile, they were totally inactive against the three tested strains of Gram-negative bacteria. Additionally, four compounds were able to exert antifungal activity against *C. albicans*, whereas, all of the tested compounds lacked antifungal activity against *Asp. niger* fungus. Potential antibacterial and antifungal activities were confined mainly to compounds comprising the thiosemicarbazide function **6a – f** at position-5, and those substituted with both the thioureido and thiosemicarbazide moieties **12a – f** at positions-2 and -5 of the thiazole ring, respectively. Compounds **6f** and **12f** (R = 4-F-$C_6H_4$) could be considered as the most active members in the present investigation with a broad spectrum potential antibacterial activity against the three types of Gram-positive bacteria tested comparable to that of ampicillin, together with a good antifungal activity against *C. albicans* comparable to that of clotrimazole. Special high activity was displayed by compounds **6d**, **6f**, and **12f** against *B. subtilis,* which were twice as active as ampicillin. The best antifungal activity was shown by compound **6d**, which was 50% less active than that of clotrimazole.

On the other hand, according to the current one-dose protocol of the NCI *in-vitro* disease-oriented human cells screening panel assay, the non-small cell lung cancer Hop-92, ovarian cancer IGROV1, and melanoma SK-MEL-2 cell lines exhibited interesting sensitivity against most of the tested 17 compounds. Compound **12f** proved to be the most active anticancer member in this study with a broad spectrum of activity against most of the tested subpanel tumor cell lines with particular effectiveness against the non-small cell lung cancer Hop-92 and melanoma SK-MEL-2 cell lines. Interestingly, compound **12f** has satisfied the pre-determined threshold inhibition criteria and consequently was carried over to be tested in the five-dose assay. Finally, such type of substituted thiazoles could be used as a template for future development through modification or derivatization in order to design more potent and selective antimicrobial and / or anticancer agents.

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*The authors have declared no conflict of interest.*

## Experimental

### Chemistry

Melting points were determined in open glass capillaries on a Gallenkamp melting point apparatus (Weiss-Gallenkamp, London, UK) and were uncorrected. The infrared (IR) spectra were recorded on Perkin-Elmer 297 infrared spectrophotometer (Perkin-Elmer, USA) using the NaCl plate technique. The $^1$H-NMR spectra were recorded on a Varian EM 360 spectrometer (Varian Inc., Palo Alto, CA, USA) using tetramethylsilane as the internal standard and DMSO-$d_6$ as the solvent (Chemical shifts in (δ, ppm). Splitting patterns were designated as follows: s: singlet; d: doublet; m: multiplet. Elemental analyses were performed at the Microanalytical Unit, Faculty of Science, King Abdul-Aziz University, Jeddah, Saudi Arabia, and the found values were within ± 0.4% of the theoretical values. Follow up of the reactions and checking the homogeneity of the compounds were made by TLC on silica gel-protected aluminum sheets (Type 60 F254, Merck, Germany) and the spots were detected by exposure to UV-lamp at $\lambda$ = 254. The synthesis of ethyl 2-amino-4-methylthiazole-5-carboxylate **1** was performed according to a reported literature procedure [36].

#### *Ethyl 2-acetylamino-4-methylthiazole-5-carboxylate **2***

The thiazole **1** (0.93 g, 5 mmol) was warmed with acetic anhydride (6 mL) for 1 h, then the mixture was allowed to attain room temperature. The deposited solid was filtered, washed with petroleum ether (60 – 80°C) dried and recrystallized. Physicochemical and analytical data are recorded in Table 3. IR (cm$^{-1}$): 3560 – 3100 (NH), 1730 (C=O), 1670 (C=O). $^1$H-NMR (δ, ppm): 1.35 (t, $J$ = 9 Hz, 3H, ester-$CH_3$), 2.15 (s, 3H, acetyl-$CH_3$), 2.62 (s, 3H, Thiazol-$C_4$-$CH_3$), 4.26 (q, $J$ = 9 Hz, 2H, ester-$CH_2$), 8.08 (brs, 1H, NH).

#### *2-Acetamido-4-methylthiazole-5-carboxylic acid hydrazide **3***

A solution of **2** (2.28 g, 10 mmol) in ethanol (20 mL) was heated under reflux with hydrazine hydrate (1.5 g, 30 mmol) for 4 h.

 

The reaction mixture was allowed to attain room temperature and the deposited solid was filtered, washed, dried, and recrystallized. Physicochemical and analytical data are recorded in Table 3. IR ($cm^{-1}$): 3750–3270 (NH), 1685 (C=O), 1645 (C=O). $^1$H-NMR (δ, ppm): 2.31 (s, 3H, $CH_3$-CO), 2.54 (s, 3H, Thiazol-$C_4$-$CH_3$), 7.68 (brs, 1H, NH), 8.10 (m, 1H, NH), 9.20 (brs, 2H, $NH_2$).

### *2-Acetamido-N-arylidine-4-methylthiazole-5-carboxylic acid hydrazides **4a–g***

A solution of **3** (1.1 g, 5 mmol) and the appropriate aldehyde (5 mmol) in glacial acetic acid (15 mL) was refluxed for 6–8 h. The solid separated upon cooling was filtered, washed with cold ethanol, dried, and recrystallized. Physicochemical and analytical data are recorded in Table 3. IR ($cm^{-1}$): 3540–2775 (NH), 1705–1645 (C=O). $^1$H-NMR (d, ppm) for **4b** (R = 4-$CH_3$-$C_6H_4$): 2.24 (s, 3H, $CH_3$-CO), 2.39 (s, 3H, $CH_3$), 2.49 (s, 3H, Thiazol-$C_4$-$CH_3$), 7.25–7.81 (m, 4H, Ar-H), 7.91 (brs, 1H, NH), 8.25 (s, 1H, NH), 8.70 (s, H, CH=N). For **4d** (R = 2-thienyl): 2.21 (s, 3H, $CH_3$-CO), 2.54 (s, 3H, Thiazol-$C_4$-$CH_3$), 7.17–7.34 (m, 3H, thiophen-3H), 8.03 (brs, 1H, NH), 8.32 (s, 1H, NH), 8.49 (s, H, CH=N). For **4h** (R = 1-morpholinyl): 2.26 (s, 3H, $CH_3$-CO), 2.64 (s, 3H, Thiazol-$C_4$-$CH_3$), 3.92 (m, 2H, morpholin-2H), 4.21 (m, 2H, morpholin-2H), 7.68 (brs, 1H, NH), 8.20 (s, 1H, NH), 8.59 (s, H, CH=N).

### *4-Substituted-1-(2-acetamido-4-methylthiazole-5-carbonyl)semicarbazides **5a, b***

To a solution of the acid hydrazide **3** (1.1 g, 5 mmol) in pyridine (15 mL), was added cyclohexyl or phenyl isocyanate (6 mmol). The reaction mixture was refluxed for 6–8 h, then allowed to attain room temperature. The reaction mixture was poured on crushed ice and the separated solid product was filtered, washed with water, dried, and recrystallized. Physicochemical and analytical data are recorded in Table 3. IR ($cm^{-1}$): 3640-2910 (NH), 1720-1630 (C=O). $^1$H-NMR (δ, ppm) for **5a** (R = cyclohexyl): 1.41–2.02 (m, 11H, cyclohexyl-H), 2.16 (s, 3H, $CH_3$-CO), 2.52 (s, 3H, Thiazol-$C_4$-$CH_3$), 5.38 (brs, 1H, NH), 7.81 (brs, 1H, NH), 8.62–9.10 (m, 2H, 2 NH). For **5b** (R = $C_6H_5$): 2.15 (s, 3H, $CH_3$-CO), 2.52 (s, 3H, Thiazol-$C_4$-$CH_3$), 5.90 (brs, 1H, NH), 7.08–7.92 (m, 6H, 5 Ar-H + NH), 8.48–9.30 (m, 2H, 2 NH).

### *4-Substituted-1-(2-acetamido-4-methylthiazole-5-carbonyl)thiosemicarbazides **6a–f***

To a solution of the acid hydrazide **3** (1.1 g, 5 mmol) in pyridine (15 mL), was added the appropriate isothiocyanate (6 mmol) and the mixture was refluxed for 4–6 h. Working up of the reaction mixture was carried out as described under **5a**, **b.** Physicochemical and analytical data are recorded in Table 3. IR ($cm^{-1}$): 3470–2935 (NH), 1640-1710 (C=O), 990–954 (NCS). $^1$H-NMR (δ, ppm) for **6a** (R = $CH_3$): 2.29 (s, 3H, $CH_3$-CO), 2.47 (s, 3H, $CH_3$), 2.53 (s, 3H, Thiazol-$C_4$-$CH_3$), 4.37–4.61 (m, 2H, 2 NH), 7.70 (brs, 1H, NH), 8.57 (brs, 1H, NH). For **6d** (R = $C_6H_5$): 2.23 (s, 3H, $CH_3$-CO), 2.57 (s, 3H, Thiazol-$C_4$-$CH_3$), 6.20–6.43 (m, 2H, 2 NH), 7.65–7.90 (m, 5H, Ar-H), 8.64 (brs, 1H, NH), 9.01 (brs, 1H, NH). For **6e** (R = 4-$CH_3$-$C_6H_4$): 2.31 (s, 3H, $CH_3$-CO), 2.46 (s, 3H, tolyl-$CH_3$), 2.55 (s, 3H, Thiazol-$C_4$-$CH_3$), 4.39–4.65 (m, 2H, 2 NH), 7.05–7.53 (m, 4H, Ar-H), 7.72 (brs, 1H, NH), 8.55 (brs, 1H, NH).

### *2-Acetamido-N-formyl-4-methylthiazole-5-carboxylic acid hydrazide **7***

A solution of the acid hydrazide **3** (1.1 g, 5 mmol) in formic acid (10 mL) was heated under reflux for 1 h, during which a solid product was partially crystallized out. After being cooled to room temperature, the product was filtered, washed, dried, and recrystallized. Physicochemical and analytical data are recorded in Table 3. IR ($cm^{-1}$): 3145–2930 (NH), 1667 (C=O aldehyde), 1630 (C=O amide). $^1$H-NMR (δ, ppm): 2.26 (s, 3H, CO-$CH_3$), 2.57 (s, 3H, Thiazol-$C_4$-$CH_3$), 5.90 (brs, 1H, NH), 8.25 (s, 1H, CHO), 9.04 (brs, 1H, NH), 10.12 (s, 1H, NH).

### *2-Acetamido-N-(4-substituted benzenesulfonyl)-4-methylthiazole-5-carboxylic acid hydrazides **8a, b***

A mixture of the start **3** (1.1 g, 5 mmol) and benzenesulfonyl chloride or tosyl chloride (5 mmol) in pyridine (10 mL), was heated under reflux for 3 h. The reaction mixture was left to attain room temperature, poured on crushed ice and the separated solid product was filtered, washed with water, dried, and recrystallized. Physicochemical and analytical data are recorded in Table 3. IR ($cm^{-1}$): 3275–2760 (NH), 1680–1690 (C=O). $^1$H-NMR (δ, ppm) for **8a** (X = H): 2.24 (s, 3H, CO-$CH_3$), 2.57 (s, 3H, Thiazol-$C_4$-$CH_3$), 7.55–7.86 (m, 6H, 5 Ar-H + NH), 8.12 (brs, 1H, NH), 7.95 (s, 1H, NH). For **8b** (X = $CH_3$): 2.30 (s, 3H, CO-$CH_3$), 2.47 (s, 3H, tolyl-$CH_3$), 2.55 (s, 3H, Thiazol-$C_4$-$CH_3$), 7.26–7.81 (m, 5H, 4 Ar-H + NH), 7.95 (brs, 1H, NH), 8.25 (s, 1H, NH).

### *2-Amino-4-methylthiazole-5-carboxylic acid hydrazide **9***

A solution of the **1** (0.93 g, 5 mmol) in ethanol (15 mL), was treated with hydrazine hydrate (0.75 g, 15 mmol) and the reaction mixture was heated under reflux for 3 h. The obtained precipitate upon cooling was filtered, washed, dried, and recrystallized. Physicochemical and analytical data are recorded in Table 3. IR ($cm^{-1}$): 3320-2760 (NH), 1670 (C=O). $^1$H-NMR (δ, ppm): 2.45 (s, 3H, Thiazol-$C_4$-$CH_3$), 6.28 (brs, 2H, $NH_2$), 8.20 (m, 1H, NH), 9.16 (brs, 2H, $NH_2$).

### *2-Acetamido-N-acetyl-4-methylthiazole-5-carboxylic acid hydrazide **10***

The acid hydrazide **9** (0.86 g, 5 mmol) was warmed with acetic anhydride (10 mL) for 2 h, then the mixture was allowed to attain room temperature. The deposited solid was filtered, washed with petroleum ether (60–80°C) and recrystallized. Physicochemical and analytical data are recorded in Table 3. IR ($cm^{-1}$): 3550–3105 (NH), 1758 (C=O acetyl), 1734 (C=O acetyl), 1667 (C=O amide). $^1$H-NMR (δ, ppm): 2.37 (s, 3H, $CH_3$), 2.49 (s, 3H, $CH_3$), 2.64 (s, 3H, Thiazol-$C_4$-$CH_3$), 5.85 (brs, 1H, NH), 8.51 (brs, 1H, NH), 9.36 (brs, 1H, NH).

### *4-Substituted-1-[2-(N-substituted ureido)-4-methylthiazol-5-carbonyl]semicarbazides **11a, b***

To a solution of the acid hydrazide **9** (0.86 g, 5 mmol) in pyridine (15 mL), was added cyclohexyl or phenyl isocyanate (12 mmol), and the mixture was refluxed for 6–8 h. Working up of the reaction mixture was carried out as described under **5a**, **b**. Physicochemical and analytical data are recorded in Table 3. IR ($cm^{-1}$): 3570-2890 (NH), 1716–1628 (C=O). $^1$H-NMR (δ, ppm) for **11a** (R = cyclohexyl): 1.36–2.05 (m, 22H, cyclohexyl-H), 2.54 (s, 3H, Thiazol-$C_4$-$CH_3$), 6.07 (brs, 1H, NH), 6.20 (brs, 1H, NH), 7.98 (brs, 1H, NH), 8.19 (brs, 1H, NH), 8.61 (brs, 1H, NH). For **11b** (R = $C_6H_5$): 2.52

 

(s, 3H, Thiazol-$C_4$-$CH_3$), 6.03 (brs, 1H, NH), 6.14 (brs, 1H, NH), 6.89 – 7.57 (m, 10H, Ar-H), 8.01 (brs, 1H, NH), 8.14 (brs, 1H, NH), 8.52 (brs, 1H, NH).

### *4-Substituted-1-[2-(N-substituted thioureido)-4-methylthiazol-5-carbonyl]thiosemicarbazides* ***12a – f***

To a solution of the acid hydrazide **9** (0.86 g, 5 mmol) in pyridine (15 mL), was added the appropriate isothiocyanate (12 mmol). The reaction mixture was refluxed for 8 – 10 h then cooled to room temperature. Working up of the reaction mixture was carried out as described under **6a–b**. Physicochemical and analytical data are recorded in Table 3. IR ($cm^{-1}$): 3520 – 2920 (NH), 1700- 1635 (C=O), 985 – 950 (NCS). $^1$H-NMR (δ, ppm) for **12a** (R = $CH_3$): 2.45 (s, 3H, Thiazol-$C_4$-$CH_3$), 2.51 (s, 3H, $CH_3$), 2.59 (s, 3H, $CH_3$), 6.03 (m, 2H, NH), 8.23 (brs, 2H, NH), 8.64 (brs, 1H, NH). For **12c** (R = $CH_2$-$C_6H_5$): 2.51 (s, 3H, Thiazol-$C_4$-$CH_3$), 4.77 (d, 2H, $CH_2$), 4.85 (d, 2H, $CH_2$), 6.11 m, 2H, NH), 7.09 – 7.66 (m, 10H, Ar-H), 8.15 (brs, 2H, NH), 8.55 (brs, 1H, NH). For **12f** (R = 4-F-$C_6H_4$): 2.49 (s, 3H, Thiazol-$C_4$-$CH_3$), 6.11 (m, 2H, NH), 6.87 – 7.71 (m, 8H, Ar-H), 8.20 (brs, 2H, NH), 8.61 (brs, 1H, NH).

## Antimicrobial screening

### *Inhibition zone (IZ) measurement*

Standard sterilized filter paper discs (5 mm diameter) impregnated with a solution of the test compound in DMSO (1 mg/mL) were placed on an agar plate seeded with the appropriate test organism in triplicates. The utilized test organisms were: *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (NRRL B-14819), and *Bacillus cereus* (ATCC 14579) as examples of Gram-positive bacteria and *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Klebsiella pneumonia* (clinical isolate) as examples of Gram-negative bacteria. *Candida albicans* (ATCC 10231) and *Aspergillus niger* (recultured) fungal strains were utilized as representatives for fungi. Ampicillin trihydrate and clotrimazole were used as standard antibacterial and antifungal agents, respectively. DMSO alone was used as control at the same abovementioned concentration. The plates were incubated at 37°C for 24 h for bacteria and for seven days for fungi. The results were recorded for each tested compound as the average diameter of inhibition zones of bacterial growth around the discs in mm (Table 1).

### *Minimal inhibitory concentration (MIC) measurement*

MICs were measured for compounds that showed significant growth inhibition zones (≥14 mm) using the two-fold serial dilution technique [37]. The microdilution susceptibility test in Müller – Hinton Broth (Oxoid) and Sabouraud Liquid Medium (Oxoid) was used for the determination of antibacterial and antifungal activity, respectively. Stock solutions of the tested compounds, ampicillin trihydrate and clotrimazole were prepared in DMSO at concentration of 800 μg/mL followed by two-fold dilution at concentrations of (400, 200, …, 6.25 μg/mL). The microorganism suspensions at $10^6$ CFU/ml (Colony Forming Unit/mL) concentration were inoculated to the corresponding wells. Plates were incubated at 36°C for 24 h to 48 h and the minimal inhibitory concentrations (MIC) were determined. Control experiments were also done. The results were recorded for each tested compound in Table 2.

## Preliminary *in-vitro* anticancer screening

Out of the newly synthesized derivatives, 17 compounds, namely **3**, **4a**, **d – h**, **5a**, **b**, **6c**, **f**, **7a**, **8a**, **10**, **11a**, **b**, **11d – h**, and **12f**; were selected by the National Cancer Institute (NCI) *in-vitro* disease-oriented human cells screening panel assay to be evaluated for their *in-vitro* anticancer activity. Primary *in-vitro* one-dose anticancer assay was performed using the full NCI 60 cell panel in accordance with the current protocol of the Drug Evaluation Branch, NCI, Bethesda [38 – 40]. These cell lines were incubated with one concentration (10 μM) for each tested compound. A 48 h continuous drug exposure protocol was used, and a sulphorhodamine B (SRB) protein assay was employed to estimate cell viability or growth.

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