European Journal of Medicinal Chemistry 76 (2014) 170–181

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Bifunctional ethyl 2-amino-4-methylthiazole-5-carboxylate derivatives: Synthesis and *in vitro* biological evaluation as antimicrobial and anticancer agents

Sherif A.F. Rostom [a,b,*], Hassan M. Faidallah [c], Mohammed F. Radwan [a,d], Mona H. Badr [a,b]

[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, King Abdulaziz University, P.O. Box 80260, Jeddah 21589, Saudi Arabia
[b] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Alexandria University, Alexandria 21521, Egypt
[c] Department of Chemistry, Faculty of Science, King Abdulaziz University, P.O. Box 80203, Jeddah 21589, Saudi Arabia
[d] Department of Medicinal Chemistry, Faculty of Pharmacy, Minia University, Minia 61519, Egypt

ARTICLE INFO

Article history:
Received 17 November 2013
Received in revised form
15 January 2014
Accepted 8 February 2014
Available online 11 February 2014

Keywords:
Synthesis
Thiazoles
Antibacterial
Antifungal
Anticancer

ABSTRACT

Thirty thiazole compounds bearing chemotherapeutically-active pharmacophores were synthesized and evaluated for their preliminary *in vitro* antimicrobial and anticancer activities. Nineteen compounds displayed obvious antibacterial potential, with special bactericidal activity against Gram positive bacteria, whereas, nine analogs showed moderate to weak antifungal activity against *Candida albicans*. The analog **12f** proved to be the most active antimicrobial member identified in this study being comparable to ampicillin and gentamicin sulfate against *Staphylococcus aureus* and *Bacillus subtilis*, together with a moderate antifungal activity. Additionally, nine derivatives were tested for their preliminary *in vitro* anticancer activity according to the current one-dose protocol of the NCI. Compound **9b** revealed a broad spectrum of anticancer activity against 29 out of the tested 60 subpanel tumor cell lines. Collectively, compounds **4**, **9b**, **10b** and **12f** could be considered as promising dual anticancer antibiotics.

1. Introduction

Human struggle against life-threatening infectious diseases brought about by multi-drug resistant (MDR) Gram-positive and Gram-negative pathogenic bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecium* (VRE), is becoming a serious global dilemma [1]. In addition, the frequent occurrence of primary and opportunistic mycotic infections (particularly *Candida albicans*) is well documented especially among immuno-compromised patients experiencing AIDS, cancer and organ transplantation [2]. Consequently, such types of infections have spurred interest in the discovery of novel non-traditional antimicrobial agents that would not induce cross-resistance with classical antibiotics. On the other hand, the high mortality rates engaged with the rising number of diverse types of cancers, have triggered an unrivaled level of research aiming at finding out new unconventional lead structures that might be beneficial in designing novel antitumor agents [3]. It is self-evident that patients with neoplastic disorders who are subjected to chemotherapeutic treatment are mostly susceptible to microbial infections due to the subsequent drop of immunity. Therefore, co-administration of multiple drugs for treating patients suffering from cancer disease accompanied with microbial infections might inflict some added health problems especially in patients with impaired liver and/or kidney functions. Therefore, the concept of monotherapy by a single drug which would possess dual utility might be advantageous from both therapeutic and cost-effective stand points.

Over the past few years, the conspicuous role of thiazole derivatives in the field of chemotherapy is unmistakable, owing to their reported distinctive antimicrobial [4–7], antifungal [8,9], antitubercular [10,11] activities. Moreover, several thiazole-containing compounds were documented to contribute to a variety of antineoplastic potentials being employed as anticancer [12–14], cytotoxic [15,16], antiproliferative [17,18], DNA-cleaving [19], anti-angiogenic [20] and tubulin polymerization inhibiting [21] agents. Recently, it has been reported that some thiazoles were stemmed as novel inhibitors of metastatic cancer cell migration and invasion [22]. Interest in the chemotherapeutic activity of thiazoles was augmented by the discovery of the natural antineoplastic antibiotic tiazofurin (**A**; Fig. 1) [23], the documented DNA minor

* Corresponding author. Department of Pharmaceutical Chemistry, Faculty of Pharmacy, King Abdulaziz University, P.O. Box 80260, Jeddah 21589, Saudi Arabia.
E-mail address: sherifrostom@yahoo.com (S.A.F. Rostom).

http://dx.doi.org/10.1016/j.ejmech.2014.02.027
0223-5234/

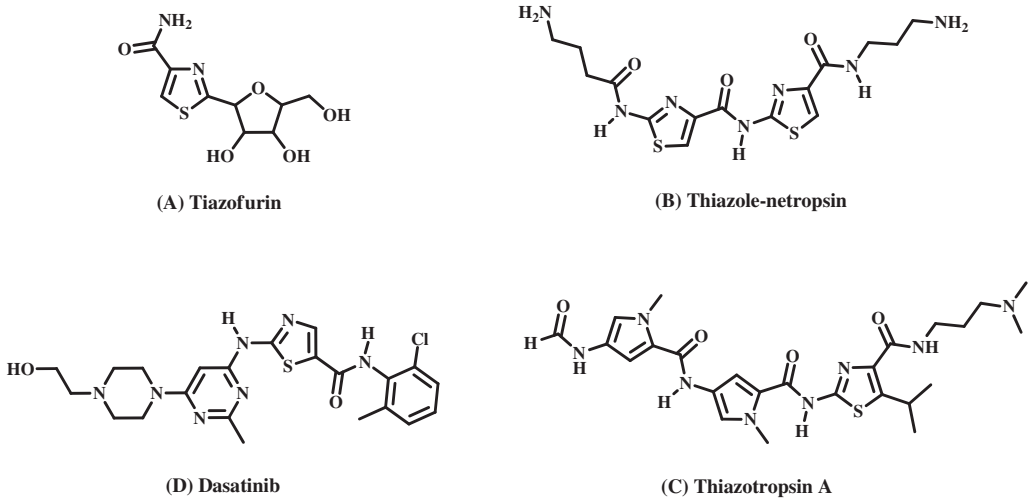


Fig. 1. Structures of tiazofurin (**A**), thiazole–netropsin (**B**), thiazotropsin A (**C**) and dasatinib (**D**).

groove binding property of thiazole–netropsin and thiazotropsin A (**B** and **C**; Fig. 1) [24,25], and the evidenced clinical antitumor effectiveness of bleomycin and leinamycin [26]. Furthermore, another thiazole derivative; dasatinib (**D**; Fig. 1) was reported to possess potential tyrosine kinase inhibitory activity, and proved to be efficient in the treatment of imatinib-resistant mutants [27]. Most of the previously-described compounds are distinguished by encountering, as a prevalent character, a substituted carboxamido functional group(s) linked to a thiazole ring.

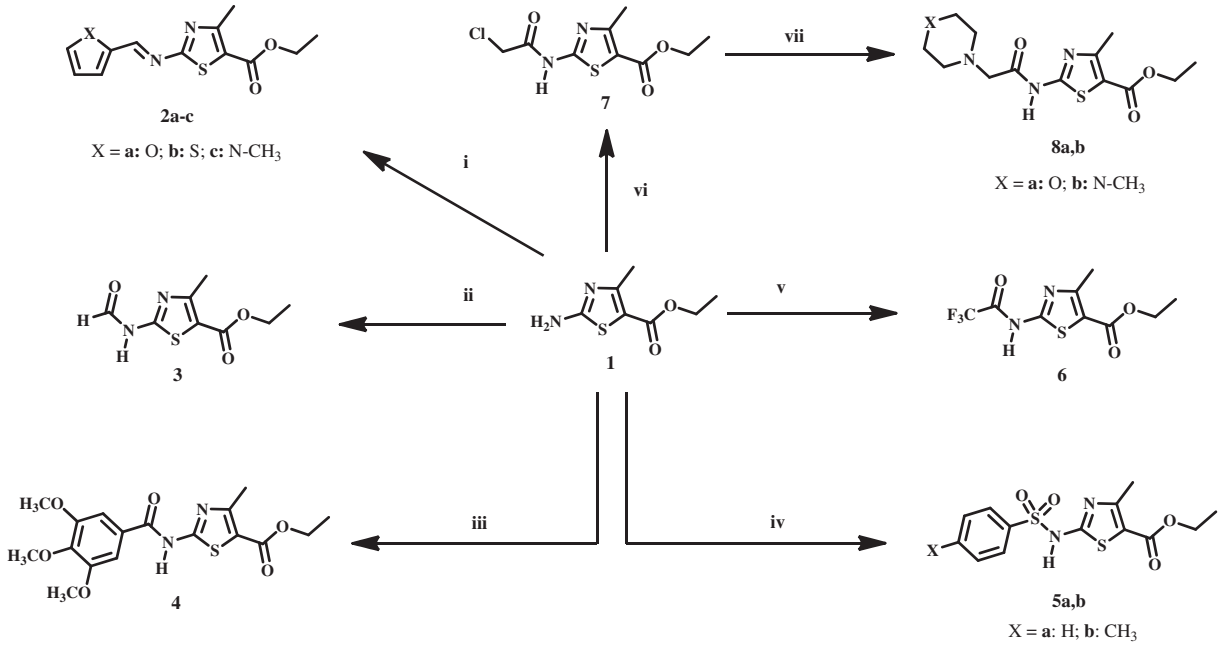
In view of the above mentioned facts, and inspired by the promising antimicrobial and anticancer activities associated with some thiazole-containing compounds reported in our previous publications [28–38], we report herein the synthesis, *in vitro* antimicrobial and anticancer evaluation of some novel bifunctional ethyl 2-amino-4-methylthiazole-5-carboxylate derivatives. The thrust of efforts in structure modification of such type of compounds focussed mainly on derivatization of the amino group at position-2 of the thiazole ring into the chemotherapeutically-active azomethine, *N*-formyl, *N*-acyl, sulfonamido, ureido and thioureido functionalities. In addition, the conversion of the ester function at position-5 to the carboxamido and acid hydrazide groups was taken into consideration based on the reported facts about their effective contribution in many potential chemotherapeutic activities [39,40]. The variability in the nature of substituents at such functionalities was attempted to represent different electronic, lipophilic and steric environment that would influence the targeted biological activities. Agar-diffusion method was used for the evaluation of the antimicrobial activity, and the minimal inhibitory (MIC, μg/mL) and minimal bactericidal (MBC, μg/mL) concentrations for the active compounds were determined [41–43]. On the other hand, the anticancer activity was evaluated according to the current protocol of the National Cancer Institute (NCI) *in vitro* disease-oriented human cells screening panel assay [44–46].

2. Results and discussion

2.1. Chemistry

Synthesis of the intermediate and target compounds **2**–**13** was performed according to the reactions illustrated in Schemes 1 and 2. The key intermediate in this study is ethyl 2-amino-4-methylthiazole-5-carboxylate **1** [47]. Condensing **1** with the appropriate heterocyclic aldehyde in acetic acid medium afforded the corresponding azomethines **2a**–**c** in good yields. Heating of the starting compound **1** with formic acid furnished the *N*-formyl derivative **3**. Its IR spectrum revealed an additional carbonyl band at 1650 cm^{-1} attributed to the new aldehyde (C=O) function, whereas the ^1H NMR spectrum (δ-ppm) exhibited a new singlet at δ 8.63 ppm due to the aldehydic proton. Furthermore, acylation of the thiazole **1** with 3,4,5-trimethoxybenzoyl chloride gave rise to the expected ethyl *N*-(3,4,5-trimethoxybenzoyl)-2-amino-4-methylthiazole-5-carboxylate **4**. Analogously, reacting **1** with benzenesulfonyl chloride or p-toluenesulfonyl chloride led to the introduction of a substituted phenylsulfonyl moiety at position-2 with the formation of the *N*-(5-ethoxycarbonyl-4-methylthiazol-2-yl)-4-substituted benzenesulfonamides **5a**,**b**. Their IR spectra showed additional bands at 1017–1110 cm^{-1} attributed to the (SO_2) function. Moreover, the 2-trifluoroacetylamino derivative **6** was successfully prepared by warming the start **1** with trifluoroacetic anhydride. On the other hand, the 2-amino function of the start **1** was alkylated with chloroacetyl chloride in dry toluene to produce the intermediate **7**. The chlorine atom of the latter was displaced by either morpholine or *N*-methyl piperazine to produce the target compounds **8a**,**b** (Scheme 1).

At this stage, some 1-(5-ethoxycarbonyl-4-methylthiazol-2-yl)-3-substituted ureas **9a**,**b** were successfully prepared by condensing the key intermediate thiazole **1** with the appropriate isocyanates in pyridine as alkaline medium. Their IR spectra showed new bands at 1645–1650 cm^{-1} corresponding to the ureido carbonyl group. In their turn, when **9a**,**b** were reacted with hydrazine hydrate, the corresponding targeted 4-methyl-2-(*N*-substituted ureido)-thiazole-5-carboxylic acid hydrazides **10a**,**b** were obtained. In an analogous fashion, when the thiazole **1** was condensed with the appropriate isothiocyanates in pyridine, the corresponding thioureas **11a**–**f** were achieved. The IR spectra of these thioureido derivatives revealed the characteristic C=S band at 982–958 cm^{-1} beside the absorption bands of the ester C=O group at 1730–1718 cm^{-1}. Similarly, the targeted 4-methyl-2-(*N*-substituted thioureido)-thiazole-5-carboxylic acid hydrazides **12a**–**f** were obtained by heating **11a**–**f** with hydrazine hydrate. The ^1H NMR spectra (δ-ppm) of the acid hydrazides **10a**,**b** and **12a**,**b** were characterized by the disappearance of the triplets and quartets of the ethyl ester and the appearance of new singlets attributed to the amides NH proton at their respective chemical shifts. Finally, refluxing the thiazole ester **1** with the appropriate amine in ethanol resulted in the formation of the corresponding



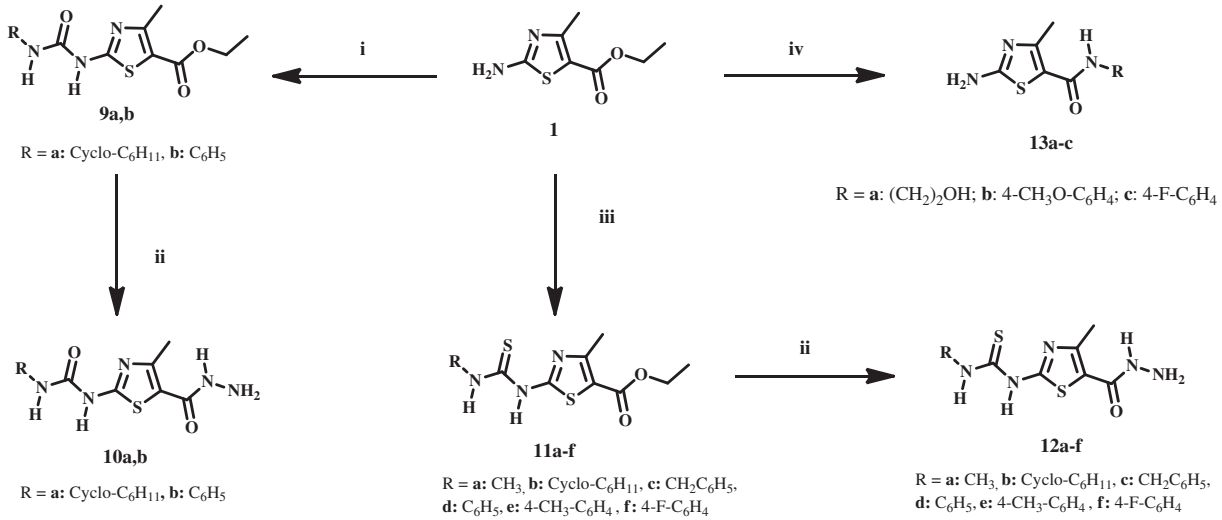
Reaction conditions: i: heterocyclic aldehydes, acetic acid, reflux, 6-8h; **ii:** HCOOH, reflux, 1h; **iii:** 3,4,5-trimethoxybenzoyl chloride, pyridine, reflux, 4h; **iv:** 4-substituted benzenesulfonylchloride, pyridine, reflux, 3h; **v:** $(CF_3CO)_2O$, warm w.b., 1h; **vi:** chloroacetyl chloride, toluene, reflux, 6h; **vii:** secondary amine, toluene, reflux, 5-8h.

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

carboxamides **13a**–**c**. Their ^{1}H NMR spectra (δ-ppm) were characterized by the disappearance of the triplet and quartet of the ethyl ester group and the appearance of a new singlet attributed to the amide NH proton at δ 8.08–8.16 ppm (Scheme 2).

2.2. In vitro antibacterial and antifungal screening

All the newly synthesized compounds were evaluated for their *in vitro* antibacterial activity against *S. aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633) as examples of Gram positive bacteria and *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) as examples of Gram negative bacteria. They were also evaluated for their *in vitro* antifungal potential against *C. albicans* (ATCC 10231) and *Aspergillus niger* (recultured) fungal strains. Agar-diffusion method was used for determination of preliminary antibacterial and antifungal activity. Ampicillin trihydrate and gentamicin sulfate were employed as standard antibacterial agents, whereas clotrimazole and amphotericin B were used as antifungal reference drugs [41]. Dimethylsulfoxide (DMSO) was used as a blank and showed no antimicrobial activity. The results were



Reaction conditions: i: substituted isocyanate, pyridine, reflux, **ii:** hydrazine hydrate 99%, ethanol, reflux, 4-6h; **iii:** substituted isothiocyanate, pyridine, reflux, 5-6h, **iv:** primary amine, ethanol, reflux, 10-12h.

Scheme 2. Synthesis of compounds **9**–**13**.

recorded for each tested compound as the average of three measurements of the diameter of inhibition zones (IZ) of bacterial or fungal growth around the cups in mm. The minimum inhibitory concentration (MIC, μg/mL) and minimum bactericidal concentration (MBC, μg/mL) measurements were determined for compounds that showed significant growth inhibition zones (≥13 mm) using the two-fold serial dilution method [42] (Table 1).

Concerning the antibacterial activity, the results revealed that 19 out of the tested 30 compounds displayed variable inhibitory effects on the growth of the tested Gram positive and Gram negative bacterial strains. In general, most of the tested compounds revealed better antibacterial activity against the Gram positive rather than the Gram negative bacteria. Among the tested Gram positive bacteria, *S. aureus* showed relative high sensitivity towards the tested compounds. In particular, compounds **4** and **12f** were equipotent to ampicillin (MIC 6.25 μg/mL) and 50% of the activity of gentamicin sulfate (MIC 3.12 μg/mL), whereas the analogs **10b**, **11f** and **13c** (MIC 12.5 μg/mL) showed half the activity of ampicillin. Moreover, compounds **5a**, **9b**, **10a** and **12e** (MIC 25 μg/mL) showed 25% of the activity of ampicillin. With regard to the activity against *B. subtilis*, compound **12f** proved to be equipotent to both ampicillin and gentamicin sulfate (MIC 12.5 μg/mL), whereas, the analogs **4**, **10b**, **11f** and **13c** (MIC 25 μg/mL) displayed half the potency of ampicillin. Meanwhile, compounds **9b** and **12e** (MIC 50 μg/mL) showed 25% of the potency of ampicillin against the same organism.

On the other hand, investigation of the antibacterial efficiency of the active compounds against the Gram negative strains revealed that three analogs namely **4**, **10b** and **12f** were able to produce noticeable growth inhibitory activity against *E. coli* (MIC 12.5 μg/mL) which represents 50% of the activity of ampicillin (MIC 6.25 μg/mL) and 25% of the activity of gentamicin sulfate (MIC 3.12 μg/mL). Whereas, compounds **2a**, **9b** and **11f** (MIC 25 μg/mL), exhibited moderate activity against the same organism when compared with ampicillin and gentamicin sulfate. On the contrary, the tested *P. aeruginosa* strain revealed moderate to weak sensitivity towards most of the active compounds (MIC range 50–100 μg/mL).

Concerning the antifungal activity of the tested compounds, only nine compounds **6**, **8a**, **9b**, **10a**, **10b**, **11f**, **12e**, **12f** and **13c** were able to induce appreciable growth inhibitory activity against *C. albicans* (MIC values 12.5–100 μg/mL, respectively) when compared with clotrimazole (MIC 6.25 μg/mL) and amphotericin B (MIC range 1.56 μg/mL), the standard antifungal agent utilized in this assay. Among these, the analog **12f** exhibited the best antifungal activity (MIC 12.5 μg/mL) that represents 50% of clotrimazole and one-eighth of that of amphotericin B. It is to be noted down that all the tested compounds lacked any antifungal activity against *A. niger*.

According to the MIC and MBC specifications of the latest National Committee on Clinical Laboratory Standards (NCCLS), it could be deduced whether the test compounds are bactericidal or bacteriostatic to the test organism [43]. In this view, as revealed from Table 1, most of the active compounds displayed bactericidal activity against most of the tested microorganisms. Concerning the Gram-positive bacteria, compounds **4**, **10b**, **11f** and **12f** were highly bactericidal against *S. aureus*, among which **4** and **12f** were equipotent to ampicillin. Compounds **10b**, **11f** and **12f** showed acceptable bactericidal activity against *B. subtilis*, especially **12f**. Meanwhile, most of the active compounds showed moderate to weak bactericidal activity against the Gram-negative *E. coli* strain, with the exception of compounds **4**, **10b** and **12f** which showed the highest bactericidal potential (50% of the activity of ampicillin). Collectively, the 1-(4-fluorophenyl)-3-(5-(hydrazinecarbonyl)-4-methylthiazol-2-yl)thiourea **12f** proved to be the most active antimicrobial member identified in this study with an activity

Table 1
Minimal inhibitory concentrations (MIC, μg/mL) and minimal bactericidal concentrations (MBC, μg/mL) of the active newly synthesized compounds.

Cpd. no.	Gram positive bacteria				Gram negative bacteria				Fungus	
	S. aureus ATCC 6538		*B. subtilis* ATCC 6633		*E. coli* ATCC 25922		*P. aeruginosa* ATCC 27853		*C. albicans* ATCC 10231	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
2a	50	100	100	200	25	50	–[a]	NT[b]	–	NT
2b	100	200	100	200	50	100	–	NT	–	NT
3	100	200	100	200	–	NT	–	NT	–	NT
4	6.25	6.25	25	50	12.5	12.5	50	100	–	NT
5a	25	25	100	100	50	50	100	200	–	NT
6	100	100	100	100	100	100	–	NT	100	100
8a	50	50	100	100	–	NT	–	NT	100	100
9a	50	50	100	100	50	100	–	NT	–	NT
9b	25	25	50	50	25	25	50	100	25	25
10a	25	50	100	100	50	100	100	200	50	100
10b	12.5	12.5	25	25	12.5	12.5	50	100	25	25
11b	50	100	100	200	50	100	–	NT	–	NT
11e	50	50	100	100	50	100	100	200	–	NT
11f	12.5	12.5	25	25	25	25	100	100	25	50
12b	100	100	100	100	50	100	100	200	–	NT
12d	50	100	100	200	100	100	–	NT	–	NT
12e	25	25	50	50	100	100	50	100	100	100
12f	6.25	6.25	12.5	12.5	12.5	12.5	50	50	12.5	12.5
13c	12.5	25	25	50	50	100	–	NT	100	100
A[c]	6.25	–	12.5	–	6.25	–	12.5	–	–	–
G[d]	3.12	–	12.5	–	3.12	–	12.5	–	–	–
C[e]	–	–	–	–	–	–	–	–	6.25	–
AB[f]	–	–	–	–	–	–	–	–	1.56	–

[a] (–): totally inactive (MIC ≥ 200 μg/mL).
[b] NT: not tested.
[c] **A**: Ampicillin trihydrate (Standard broad spectrum antibiotic).
[d] **G**: Gentamycin (Standard broad spectrum antibiotic).
[e] **C**: Clotrimazole (Standard broad spectrum antifungal agent).
[f] **AB**: Amphotericin-B (Standard broad spectrum antifungal agent).

equivalent to ampicillin and 50% of gentamicin sulfate against *S. aureus*, in addition to being equipotent to both ampicillin and gentamicin against *B. subtilis* together with a moderate antifungal activity comparable with clotrimazole and amphotericin B.

2.3. In vitro anticancer screening

2.3.1. Primary in vitro one-dose assay

Out of the newly synthesized compounds, nine derivatives **4**, **5a**, **9a**,**b**, **10a**,**b**, **12f** and **13a**,**c**; 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 turn-around time to suppliers while maintaining efficient identification of active compounds [44–46]. 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 5-dose screen. The threshold inhibition criteria for progression to the 5-dose screen were 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 the non-small cell lung cancer subpanel, the Hop-92 cell line exhibited a variable degree of sensitivity towards six compounds (**4**, **9a**, **9b**, **10a**, **12f** and **13a**), with special emphasis on compounds **10a** and **13a** which displayed percentage growth inhibition (GI%) of 82.8 and 82.1, respectively. Within the same subpanel, the growth of the NCI-H522 cell line was affected by the presence of six compounds (**4**, **9b**, **10a**, **10b**, **13a** and **13c**), among which compounds **10a**, **10b** and **13a** showed the highest growth inhibitory potential (99.5, 63.4 and 65.4%, respectively). Furthermore, among the melanoma subpanel, the growth of the SK-MEL-2 cell line proved to be noticeably affected by the presence of the active nine tested compounds, with particular sensitivity to the analogs **5a** and **13a** which displayed remarkably high GI% (97.5 and 87.4, respectively). Moreover, the growth of the ovarian cancer IGROV1 cell line was found to be inhibited by five out of the tested compounds, with an obvious sensitivity towards compounds **5a** and **9b** (GI% 93.1 and 72.9%, respectively). Referring to the colon cancer subpanel, compounds **4** and **5a** showed a

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

Cell lines	4	5a	9a	9b	10a	10b	12f	13a	13c
NSCLC[b]									
HOP-92	53.1	–[c]	53.9	64.2	82.8	–	33.7	82.1	–
NCI-H226	12.0	–	9.89	11.3	–	2.53	5.35	3.91	5.62
NCI-H322M	–	–	–	–	–	22.0	15.1	–	2.56
NCI-H522	32.6	–	–	2.64	99.5	63.4	–	65.4	9.55
Colon cancer									
HCC-2998	42.7	72.5	–	27.4	–	6.85	10.5	–	11.8
HCT-116	4.01	43.1	14.2	16.3	16.3	10.5	7.87	2.49	–
HCT-15	5.30	–	14.3	–	9.55	9.63	–	–	–
HT29	–	–	15.5	24.1	5.51	–	–	–	–
SW-620	–	–	7.50	2.85	–	–	6.48	–	–
Breast cancer									
HS 578T	11.2	–	8.40	6.40	–	2.83	2.23	–	14.2
MCF7	12.5	–	12.7	7.17	–	2.96	–	2.13	2.21
NCI/ADR-RES	4.66	–	8.96	13.9	3.01	6.43	10.0	–	2.36
MDA-MB-468	8.82	–	–	18.4	3.73	–	5.50	3.17	9.63
T-47D	–	–	4.11	12.6	–	–	2.52	–	–
Ovarian cancer									
IGROV1	–	93.1	43.7	72.9	28.0	–	6.51	–	–
Leukemia									
CCRF-CEM	11.7	–	6.76	22.0	–	–	–	40.0	–
K-562	49.0	–	9.69	39.0	–	–	–	–	–
MOLT-4	12.4	24.4	12.3	23.7	–	–	–	–	–
RPMI-8226	17.0	–	4.13	22.8	–	–	–	–	–
SR	21.0	–	35.3	14.3	–	25.8	–	–	–
Renal cancer									
A 498	11.4	11.6	15.3	28.9	–	3.17	–	–	3.67
SN 12C	7.38	–	5.62	4.19	–	–	2.43	–	3.01
UO-31	24.3	13.0	10.6	16.1	16.0	28.0	11.0	20.0	11.8
Melanoma									
LOX IMVI	3.70	–	13.0	4.13	7.54	10.1	6.73	–	–
SK-MEL-2	69.1	97.5	64.1	77.6	29.3	37.8	30.0	87.4	67.3
SK-MEL-5	5.59	–	8.95	4.58	–	2.35	5.01	–	6.65
UACC-62	11.5	–	26.0	20.0	10.2	11.9	10.8	7.33	3.24
Prostate cancer									
PC-3	35.0	–	–	4.18	–	–	2.36	–	–
CNS cancer									
SF-268	3.75	–	12.6	4.88	–	–	–	2.48	–
SNB-75	2.51	3.91	–	47.2	–	–	7.19	–	5.40
U251	–	–	–	9.58	2.59	–	5.41	–	5.77

[a] The data obtained from NCI's *in vitro* disease-oriented human tumor cell screen at 10^{-5} μM concentration.
[b] NSCLC: non-small cell lung cancer.
[c] Not active (GI% < 10).

noticeable growth inhibitory activity against the HCC-2998 cell line (GI% of 42.7 and 72.5, respectively). Although all of the tested compounds were able to inhibit the growth of the renal UO-31 cell line, yet their potential was clearly low (GI% range 10.6–28.0). On the contrary, most of the breast, prostate and CNS cancer cell lines utilized were resistant to the nine active compounds.

Further interpretation of the results revealed that, compound **9b** proved to possess a broad spectrum of anticancer activity against 29 of the tested 60 subpanel tumor cell lines, with particular effectiveness against the non-small cell lung cancer Hop-92, ovarian cancer IGROV1 and melanoma SK-MEL-2 cell lines (GI% 64.2, 72.9 and 77.6, respectively). Here it should be pointed out that, although the antitumor spectrum of analog **5a** is relatively narrow when compared with its congeners **4**, **9a**,**b**, **10a**,**b**, **12f** and **13a**,**c**, yet it revealed remarkable growth inhibitory potentials against colon cancer HCC-2998, ovarian cancer IGROV1 and melanoma SK-MEL-2 cell lines (GI% 72.5, 93.5 and 97.5, respectively). However, the aforementioned compounds displayed a promising broad spectrum antitumor profile against some of the tested subpanel tumor cell lines that would deserve further derivatization and investigation.

2.4. Structure–activity correlation

A close examination of the structures of the active antimicrobial compounds revealed that, their antimicrobial activity (Table 1) is strongly bound to the nature of the substituent at the thiazole-C_2. In Scheme 1, the azomethins **2a**–**c** showed moderate to weak antimicrobial activity against *S. aureus* and *E. coli*, among which compound **2a** (R = 2-furyl) was the most active member. Formylation of the amino group as in compound **3** led to a great reduction of the antimicrobial activity. However, increasing the lipophilicity through the formation of the 3,4,5-trimethoxybenzoylamino analog **4** resulted in a remarkable enhancement in both the potency and spectrum of the antibacterial activity, especially against *S. aureus* (MIC 6.25 μg/mL, equipotent to ampicillin). Whereas, introduction of a benzenesulfonyl moiety (**5a**; R = H) led to an obvious reduction of the overall activity (compared with the analog **4**). Acylation of the 2-amino group with either a trifluoroacetyl group or a 2-substituted acetyl moiety (as in **6** and **8a**, respectively) did not offer a significant advantage to the antimicrobial efficiency. Shifting to target compounds **9**–**13** presented in Scheme 2, derivatization of the key intermediate **1** into the 2-(3-substituted)ureido derivatives gave rise to two active compounds **9a**,**b**, among which the aromatic analog **9b** (R = C_6H_5) exhibited a noticeable broad spectrum antibacterial efficiency against both Gram positive and negative bacteria, in addition to a recognizable antifungal activity. Converting the latter ureido analog **9b** into its corresponding carboxylic acid hydrazide **10b** resulted in a remarkable improvement in the overall antimicrobial potential especially against *S. aureus*, *B. subtilis* and *E. coli*. On the other hand, bioisosteric replacement of the ureido with a thioureido functionality substituted with a variety of alkyl, aralkyl and aryl moieties, resulted in compounds **11a**–**f**, among which, the analogs **11e**,**f** with aryl substituents (R = 4-CH_3–C_6H_4 and 4-F–C_6H_4, respectively) exhibited better antimicrobial activity than the aliphatic-substituted analog **11b** (R = cyclo-C_6H_{11}). Further derivatization of the latter compounds into the targeted carboxylic acid hydrazides **12a**–**f** led to an obvious increase in the antimicrobial potential and spectrum. The 4-fluorophenyl moiety (**12f**; R = 4-F–C_6H_4) proved to be the most favorable substituent among this series as it exhibited broad spectrum antibacterial potential against all the tested Gram positive and negative strains, with special efficacy against *S. aureus* and *B. subtilis* (MIC 6.25and 12.5 μg/mL, respectively) equivalent to ampicillin, in addition to a remarkable antifungal activity against *C. albicans* (MIC 12.5 μg/mL) comparable to that of clotrimazole (MIC 6.25 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* together with a weak antifungal effect, when compared with **12f**. Finally, derivatization of the ethyl carboxylate group at C_5 into the substituted carboxamido functionality afforded one moderately active compound **13c** (R = 4-F–C_6H_4), especially against *S. aureus* and *B. subtilis*.

On the other hand, taking the spectrum of antitumor activity (Table 2) as a reliable criterion for comparison of the active anticancer analogs in the present study, it could be clearly recognized that better cell growth inhibitory activity could be correlated with derivatization of the 2-amino group, rather than the conversion of the ester function into carboxamido and acid hydrazide moieties. In this context, the trimethoxybenzamido compound **4** exhibited a reliable anticancer spectrum with a special potency against melanoma SK-MEL-2 cell line (GI% 69.1). Introduction of a sulfonamide function as in **5a** resulted in an obvious reduction in the overall activity, nevertheless, it proved prominently active against some individual cell lines such as the colon HCC-2998, ovarian IGROV1 and melanoma SK-MEL-2 cell lines (GI% 72.5, 93.1 and 97.5, respectively). Furthermore, derivatization of the 2-amino group into a substituted ureido functionality as in **9a** and **9b** showed remarkable enhancement in activity against a wide range of subpanel tumor cell lines especially leukemia and melanoma subpanels. However, further derivatization of compounds **9a**,**b** into their corresponding acid hydrazides **10a**,**b** led to a noticeable reduction in the overall anticancer spectrum, with exceptional distinctive growth inhibitory activity for **10a** against NSCLC HOP-92 and NCI-H522 cell lines (GI% 82.8 and 99.5, respectively). Bioisosteric replacement of the ureido group with a thioureido one as in **12f** led to an obvious reduction in the anticancer potency. Finally, conversion of the ester function into a substituted carboxamido group as in **13a** and **13c**, led to an overall remarkable reduction in activity. These findings are in line with literature reports which have discussed the anticancer activity of structurally relevant polysubstituted thiazoles [48–51].

A thorough literature survey revealed that the exact mechanism of anticancer activity of thiazole carboxamide derivatives is still uncertain. In addition to the previously-mentioned DNA minor groove binding property of thiazole–netropsin, thiazotropsin bleomycin and leinamycin [24–26] and the potential tyrosine kinase inhibitory activity of dasatinib [27], it was reported that the anticancer potential of such type of thiazoles might be attributed to their ability to inhibit necroptosis [52], induce apoptosis [53] and inhibit dihydrofolic acid reductase (DHFR) enzyme [54]. Moreover, some thiazole carboxamide derivatives have displayed different affinities for many cancer biotargets including non-membrane protein tyrosine phosphatase (SHP-2) [55] and JNK-stimulating phosphatase-1 (JSP-1) [56].

3. Conclusion

In conclusion, the aim of the present investigation was to synthesize novel bifunctional thiazole lead compounds as potential anticancer antibiotics. Such objective has been achieved by the synthesis of thirty compounds derived from ethyl 2-amino-4-methylthiazole-5-carboxylate supported with chemotherapeutically-active pharmacophores such as the azomethine, *N*-formyl, *N*-acyl, sulfonamido, ureido, thioureido, carboxamido and acid hydrazide functionalities. In general, better biological activity seemed to be associated with derivatization of the 2-amino group, rather than the conversion of the ester function into carboxamido and acid hydrazide moieties. The results of the antimicrobial screening revealed that nineteen derivatives displayed an obvious antibacterial activity, with special bactericidal effect on the Gram positive bacteria. Among these, compounds **4**, **9b**, **10b**, **11f** and **12f** revealed remarkable broad spectrum antibacterial profiles. Meanwhile, nine compounds were able to exhibit moderate to weak

antifungal activity against *C. albicans*, especially compounds **9b**, **10b**, **11f** and **12f**. The thioureido analog **12f** stemmed as the most active antimicrobial member identified in this study with an activity equivalent to ampicillin and 50% of gentamicin sulfate against *S. aureus*, in addition to being equipotent to both ampicillin and gentamicin against *B. subtilis*, together with a moderate antifungal activity comparable with clotrimazole and amphotericin B.

On the other hand, nine derivatives **4**, **5a**, **9a**,**b**, **10a**,**b**, **12f** and **13a**,**c** were subjected for preliminary *in vitro* anticancer screening according to the current one-dose protocol of the NCI. Five cell lines namely; the non-small cell lung cancer Hop-92, NCI-H522, ovarian cancer IGROV1, colon cancer HCC-2998 and melanoma SK-MEL-2 exhibited remarkable sensitivity against most of the tested compounds. Compound **9b** proved to possess a broad spectrum of anticancer activity against 29 of the tested 60 subpanel tumor cell lines. Whereas, compounds **4**, **5a**, **9a**, **10a**,**b**, **13a** and **13c** displayed promising antitumor profiles against particular subpanel tumor cell lines. Collectively, compounds **4**, **9b**, **10b** and **12f** (Fig. 2) could be considered as promising dual anticancer antibiotics that deserve further investigation and derivatization in order to explore the scope and limitation of their biological activities.

4. Experimental

4.1. Chemistry

Melting points were determined in open glass capillaries on a Gallenkamp melting point apparatus and were uncorrected. The infrared (IR) spectra were recorded on Perkin–Elmer 297 infrared spectrophotometer using the NaCl plate technique. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian EM 360 spectrometer 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) and the spots were detected by exposure to UV-lamp at λ 254. The synthesis of ethyl 2-amino-4-methylthiazole-5-carboxylate **1** was performed according to a reported literature procedure [47].

4.1.1. Ethyl 2-(2-arylideneamino)-4-methylthiazole-5-carboxylates (**2a**–**c**)

A solution of **1** (0.93 g, 5 mmol) and the appropriate aldehyde (5 mmol) in gl. acetic acid (15 mL) was refluxed for 6–8 h. The solid product separated upon cooling was filtered, washed with cold ethanol, dried and recrystallized. Physicochemical and analytical data are recorded in Table 3. IR (cm^{-1}): 1690–1675 (C=O), 1640–1630 (C=N).

*4.1.1.1. Ethyl 2-((furan-2-ylmethylene)amino)-4-methylthiazole-5-carboxylate (**2a**).* ^{1}H NMR (δ-ppm): 1.34 (t, J = 9 Hz, 3H, ester–CH_3), 2.53 (s, 3H, thiazol–C_4–CH_3), 4.28 (q, J = 9 Hz, 2H, ester–CH_2), 6.49–6.75 (m, 3H, furan–3H), 8.01 (s, 1H, CH=N). ^{13}C NMR (δ-ppm): 6.9 (CH_3), 13.6 (CH_3), 59.1 (CH_2), 110.5, 143.6 (Furan C), 103.2, 149.3, 171.2 (Thiazole C), 167.3 (CO), 163.4 (C=N).

*4.1.1.2. Ethyl 4-methyl-2-((thiophen-2-ylmethylene)amino)thiazole-5-carboxylate (**2b**).* ^{1}H NMR (δ-ppm): 1.36 (t, J = 9 Hz, 3H, ester–CH_3), 2.56 (s, 3H, thiazol–C_4–CH_3), 4.32 (q, J = 9 Hz, 2H, ester-–CH_2), 7.20–7.44 (m, 3H, thiophen–3H), 8.12 (s, 1H, CH=N). ^{13}C NMR (δ-ppm): 6.9 (CH_3), 13.6 (CH_3), 59.2 (CH_2), 126.4, 128.3 (Thiophene C), 103.1, 149.2, 171.3 (Thiazole C), 167.2 (CO), 163.3 (C=N).

*4.1.1.3. Ethyl 4-methyl-2-(((1-methyl-1H-pyrrol-2-yl)methylene) amino)thiazole-5-carboxylate (**2c**).* ^{1}H NMR (δ-ppm): 1.31 (t, J = 9 Hz, 3H, ester–CH_3), 2.49 (s, 3H, thiazol–C_4–CH_3), 3.54 (s, 3H, Pyrrol–CH_3), 4.23 (q, J = 9 Hz, 2H, ester–CH_2), 6.17–6.46 (m, 3H, Pyrrol–3H), 8.06 (s, 1H, CH=N). ^{13}C NMR (δ-ppm): 6.8 (CH_3), 13.4 (CH_3), 32.3 (N–CH_3), 59.0 (CH_2), 108.8, 123.1 (Pyrrole C), 103.2, 149.4, 171.5 (Thiazole C), 167.0 (CO), 163.5 (C=N).

4.1.2. Ethyl 2-formamido-4-methylthiazole-5-carboxylate (**3**)

A solution of the thiazole **1** (0.93 g, 5 mmol) in formic acid (5 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 with water, dried and recrystallized. Physicochemical and analytical data are recorded in Table 3. IR (cm^{-1}): 3145 (NH), 1690 (C=O), 1650 (C=O). ^{1}H NMR (δ-ppm): 1.41 (t, J = 9 Hz, 3H, ester–CH_3), 2.59 (s, 3H, thiazol–C_4–CH_3), 4.33 (q, J = 9 Hz, 2H, ester–CH_2), 8.12 (brs, 1H, NH), 8.63 (s, 1H, CHO). ^{13}C NMR (δ-ppm): 6.9 (CH_3), 13.7 (CH_3), 59.1 (CH_2), 103.3, 149.1, 172.0 (Thiazole C), 160.5 (CO), 167.1 (CO).

4.1.3. Ethyl 4-methyl-2-(3,4,5-trimethoxybenzamido)thiazole-5-carboxylate (**4**)

A mixture of **1** (0.93 g, 5 mmol) and 3,4,5-trimethoxybenzoyl chloride (1.15 g, 5 mmol) in pyridine (10 mL) was heated under reflux for 4 h. The reaction mixture was left to attain room temperature, poured onto crushed ice and the separated solid product was filtered, washed with water, dried and recrystallized.

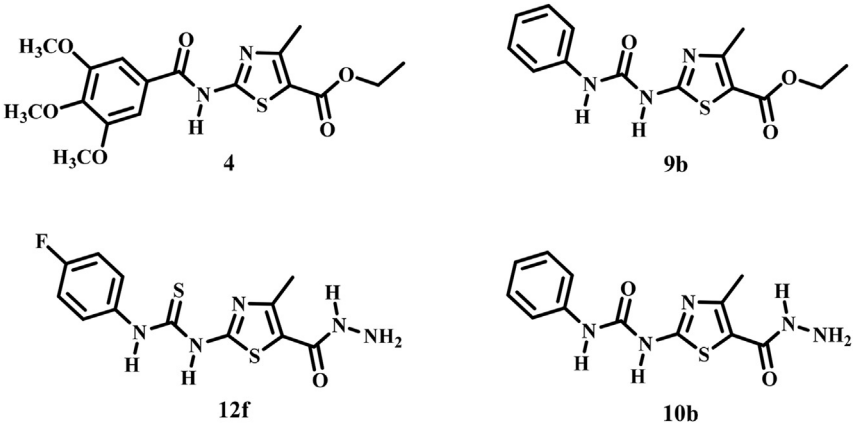


Fig. 2. Structures of the most active compounds **4**, **9b**, **10b** and **12f**.

Table 3
Physicochemical and analytical data for compounds **2**–**13**.

Cpd no.	X or R	Yield (%)	M.p. (°C) (Cryst. solv.)[a]	Mol. form. (M. wt.)		Analysis[b]		
						C	H	N
2a	O	76	201–3 (E)	$C_{12}H_{12}N_2O_3S$ (264.3)	C	54.53	4.58	10.60
					F	54.42	4.73	10.38
2b	S	89	217–8 (E)	$C_{12}H_{12}N_2O_2S_2$ (280.37)	C	51.41	4.31	9.99
					F	51.66	4.12	10.08
2c	N–CH_3	68	182–4 (E)	$C_{13}H_{15}N_3O_2S$ (277.34)	C	56.30	5.45	15.15
					F	56.57	5.33	14.91
3	–	37	175–7 (M)	$C_8H_{10}N_2O_3S$ (214.24)	C	44.85	4.70	13.08
					F	44.62	4.97	13.32
4	–	43	142–4 (E/PE)	$C_{17}H_{20}N_2O_6S$ (380.42)	C	53.67	5.30	7.36
					F	53.34	5.41	7.47
5a	H	48	177–9 (DMF/E)	$C_{13}H_{14}N_2O_4S_2$ (326.39)	C	47.84	4.32	8.58
					F	47.63	4.40	8.27
5b	CH_3	37	160–2 (DMF/E)	$C_{14}H_{16}N_2O_4S_2$ (340.42)	C	49.40	4.74	8.23
					F	49.31	4.96	8.46
6	–	78	231–3 (E/PE)	$C_9H_9F_3N_2O_3S$ (282.24)	C	38.30	3.21	9.93
					F	38.09	3.38	10.11
7	–	84	102–4 (E/W)	$C_9H_{11}ClN_2O_3S$ (262.71)	C	41.15	4.22	10.66
					F	40.91	4.43	10.75
8a	O	59	122–4 (E)	$C_{13}H_{19}N_3O_4S$ (313.37)	C	49.83	6.11	13.41
					F	49.97	6.03	13.78
8b	N–CH_3	48	147–9 (E)	$C_{14}H_{22}N_4O_3S$ (326.41)	C	51.51	6.79	17.16
					F	51.74	6.88	16.98
9a	Cyclo-C_6H_{11}	86	163–5 (E)	$C_{14}H_{21}N_3O_3S$ (311.40)	C	54.00	6.80	13.49
					F	54.31	6.63	13.37
9b	C_6H_5	76	254–6 (E)	$C_{14}H_{15}N_3O_3S$ (305.35)	C	55.07	4.95	13.76
					F	54.83	5.09	13.53
10a	Cyclo-C_6H_{11}	53	170–1 (E)	$C_{12}H_{19}N_5O_2S$ (297.38)	C	48.47	6.44	23.55
					F	48.26	6.61	23.72
10b	C_6H_5	41	230–2 (E)	$C_{12}H_{13}N_5O_2S$ (291.33)	C	49.47	4.50	24.04
					F	49.56	4.42	23.89
11a	CH_3	37	158–9 (E)	$C_9H_{13}N_3O_2S_2$ (259.35)	C	41.68	5.05	16.20
					F	41.46	5.30	16.47
11b	Cyclo-C_6H_{11}	62	166–7 (E)	$C_{14}H_{21}N_3O_2S_2$ (327.47)	C	51.35	6.46	12.83
					F	51.47	6.60	12.96
11c	C_6H_5– CH_2	60	141–3 (E/W)	$C_{15}H_{17}N_3O_2S_2$ (335.44)	C	53.71	5.11	12.53
					F	53.59	5.43	12.77
11d	C_6H_5	50	164–6 (E)	$C_{14}H_{15}N_3O_2S_2$ (321.42)	C	52.32	4.70	13.07
					F	52.54	4.51	12.91
11e	4-CH_3-C_6H_4	80	170–2 (E)	$C_{15}H_{17}N_3O_2S_2$ (335.45)	C	53.71	5.11	12.53
					F	53.46	5.49	12.71
11f	4-F-C_6H_4	78	178–0 (E)	$C_{14}H_{14}FN_3O_2S_2$ (339.41)	C	49.54	4.16	12.38
					F	49.80	4.03	12.27
12a	CH_3	19	186–8 (E/PE)	$C_7H_{11}N_5OS_2$ (245.33)	C	34.27	4.52	28.55
					F	34.13	4.64	28.31
12b	Cyclo-C_6H_{11}	27	197–9 (E)	$C_{12}H_{19}N_5OS_2$ (313.44)	C	45.98	6.11	22.34
					F	46.11	6.03	22.46
12c	C_6H_5– CH_2	21	146–8 (E/W)	$C_{13}H_{15}N_5OS_2$ (321.42)	C	48.58	4.70	21.79
					F	48.69	4.43	21.56
12d	C_6H_5	34	264–6 (E)	$C_{12}H_{13}N_5OS_2$ (307.39)	C	46.89	4.26	22.78
					F	47.04	4.13	22.91
12e	4-CH_3-C_6H_4	28	284–6 (E)	$C_{13}H_{15}N_5OS_2$ (321.43)	C	48.58	4.70	21.79
					F	48.64	4.51	21.61
12f	4-F-C_6H_4	27	297–9 (E/W)	$C_{12}H_{12}FN_5OS_2$ (325.38)	C	44.29	3.72	21.52
					F	44.36	3.63	21.27
13a	CH_2CH_2OH	31	191–3 (D/W)	$C_7H_{11}N_3O_2S$ (201.25)	C	41.78	5.51	20.88
					F	41.90	5.38	20.61
13b	4-CH_3O–C_6H_4	25	148–2 (E)	$C_{12}H_{13}N_3O_2S$ (263.32)	C	54.74	4.98	15.96
					F	54.81	5.02	15.74
13c	4-F-C_6H_4	27	176–7 (E)	$C_{11}H_{10}FN_3OS$ (251.28)	C	52.58	4.01	16.72
					F	52.70	3.88	16.91

[a] Crystallization solvent(s): A: acetic acid, D: dioxane, DMF: *N,N*-dimethylformamide, E: ethanol, M: methanol, PE: petroleum ether (60:80), W: water.
[b] The found values (F) are within ±0.4% of the calculated (C) values.

Physicochemical and analytical data are recorded in Table 3. IR (cm^{-1}): 3225 (NH), 1715 (C=O), 1680 (C=O). ^1H NMR (δ-ppm): 1.37 (t, J = 9 Hz, 3H, ester–CH_3), 2.49 (s, 3H, Thiazol–C_4–CH_3), 3.89 (s, 6H, 2OCH_3), 4.06 (s, 3H, OCH_3), 4.28 (q, J = 9 Hz, 2H, ester–CH_2), 6.79 (s, 1H, Ar–H), 6.90 (s, 1H, Ar–H), 8.11 (brs, 1H, NH). ^{13}C NMR (δ-ppm): 6.8 (CH_3), 13.6 (CH_3), 56.3 (CH_3O), 56.6 (CH_3O), 59.0 (CH_2), 103.2, 149.0, 171.7 (Thiazole C), 106.2, 127.8, 136.5, 148.6 (Ar C), 165.2 (CO), 167.0 (CO).

*4.1.4. Ethyl 4-methyl-2-(4-substituted phenylsulfonamido)thiazole-5-carboxylates (**5a**,**b**)*

A mixture of the start **1** (0.93 g, 5 mmol) and benzenesulfonyl chloride or tosyl chloride (5 mmol) in pyridine (10 mL) was heated under reflux for 3 h. Working up of the reaction mixture was carried out as described under **4**. Physicochemical and analytical data are recorded in Table 3. IR (cm^{-1}): 3380–3125 (NH), 1690–1680 (C=O), 1110–1017 (SO_2).

*4.1.4.1. Ethyl 4-methyl-2-(phenylsulfonamido)thiazole-5-carboxylate (**5a**).* ^{1}H NMR (δ-ppm): 1.31 (t, J = 9 Hz, 3H, ester–CH_3), 2.51 (s, 3H, Thiazol–C_4–CH_3), 4.26 (q, J = 9 Hz, 2H, ester–CH_2), 7.42–7.71 (m, 5H, Ar–H), 8.13 (brs, 1H, NH). ^{13}C NMR (δ-ppm): 6.9 (CH_3), 13.1 (CH_3), 59.0 (CH_2), 103.8, 149.2, 171.9 (Thiazole C), 125.5, 128.8, 131.6, 139.3 (Ar C), 165.1 (CO), 167.2 (CO).

*4.1.4.2. Ethyl 4-methyl-2-(4-methylphenylsulfonamido)thiazole-5-carboxylate (**5b**).* ^{1}H NMR (δ-ppm): 1.29 (t, J = 9 Hz, 3H, ester–CH_3), 2.36 (s, 3H, tolyl–CH_3), 2.47 (s, 3H, Thiazol–C_4–CH_3), 4.24 (q, J = 9 Hz, 2H, ester–CH_2), 7.23–7.70 (m, 4H, Ar–H), 7.86 (brs, 1H, NH). ^{13}C NMR (δ-ppm): 6.9 (CH_3), 13.5 (CH_3), 20.9 (CH_3), 59.0 (CH_2), 104.0, 149.3, 172.1 (Thiazole C), 125.4, 129.3, 136.2, 141.0 (Ar C), 165.2 (CO), 167.0 (CO).

*4.1.5. Ethyl 4-methyl-2-(2,2,2-trifluoroacetamido)thiazole-5-carboxylate (**6**)*

The thiazole **1** (0.93 g, 5 mmol) was warmed with trifluoroacetic anhydride (6 mL) on a water bath 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}): 3330 (NH), 1730 (C=O), 1670 (C=O). ^{1}H NMR (δ-ppm): 1.37 (t, J = 9 Hz, 3H, ester–CH_3), 2.59 (s, 3H, Thiazol–C_4–CH_3), 4.27 (q, J = 9 Hz, 2H, ester–CH_2), 8.08 (brs, 1H, NH). ^{13}C NMR (δ-ppm): 6.9 (CH_3), 13.3 (CH_3), 59.4 (CH_2), 124.9 (CF_3), 103.6, 149.3, 172.1 (Thiazole C), 160.4 (CO), 167.0 (CO).

*4.1.6. Ethyl 2-(2-chloroacetamido)-4-methylthiazole-5-carboxylate (**7**)*

Chloroacetyl chloride (0.85 g, 7.5 mmol) was added dropwise to a stirred solution of the thiazole **1** (0.93 g, 5 mmol) in dry toluene (15 mL) at room temperature. The reaction mixture was heated under reflux for 6 h, then the solvent was removed under reduced pressure and the remaining pale yellow residue was treated with water, filtered, dried and recrystallized. Physicochemical and analytical data are recorded in Table 3. IR (cm^{-1}): 3215 (NH), 1690 (C=O), 1645 (C=O). ^{1}H NMR (δ-ppm): 1.34 (t, J = 9 Hz, 3H, ester–CH_3), 2.61 (s, 3H, Thiazol–C_4–CH_3), 4.29 (q, J = 9 Hz, 2H, ester–CH_2), 4.41 (s, 2H, CH_2–Cl), 8.12 (brs, 1H, NH). ^{13}C NMR (δ-ppm): 6.8 (CH_3), 13.4 (CH_3), 48.8 (CH_2Cl), 59.0 (CH_2), 104.0, 149.3, 172.1 (Thiazole C), 163.2 (CO), 168.1 (CO).

*4.1.7. Ethyl 4-methyl-2-(2-(substituted amino)acetamido)thiazole-5-carboxylates (**8a**,**b**)*

To a stirred solution of **7** (1 g, 4 mmol) in dry toluene (20 mL), the appropriate secondary amine (8 mmol) was added dropwise. The reaction mixture was heated under reflux for 5–8 h and the solvent was removed under reduced pressure. The obtained residue was treated with ice-cold water, filtered, dried and recrystallized. Physicochemical and analytical data are recorded in Table 3. IR (cm^{-1}): 3430–3175 (NH), 1690–1681 (C=O), 1655–1650 (C=O).

*4.1.7.1. Ethyl 4-methyl-2-(2-morpholinoacetamido)thiazole-5-carboxylate (**8a**).* ^{1}H NMR (δ-ppm): 1.36 (t, J = 9 Hz, 3H, ester–CH_3), 2.34–2.53 (m, 4H, morpholine–H), 2.64 (s, 3H, Thiazol–C_4–CH_3), 3.46–3.67 (m, 4H, morpholine–H), 4.36 (q, J = 9 Hz, 2H, ester–CH_2), 4.46 (s, 2H, CH_2), 8.09 (brs, 1H, NH). ^{13}C NMR (δ-ppm): 7.0 (CH_3), 13.6 (CH_3), 59.1 (CH_2), 55.9 (morpholine α-C), 71.1 (morpholine β-C), 103.6, 149.5, 172.0 (Thiazole C), 167.1 (CO), 168.2 (CO).

*4.1.7.2. Ethyl 4-methyl-2-(2-(4-methylpiperazin-1-yl)acetamido) thiazole-5-carboxylate (**8b**).* ^{1}H NMR (δ-ppm): 1.38 (t, J = 9 Hz, 3H, ester–CH_3), 2.19 (s, 3H, N–CH_3), 2.29–2.63 (m, 8H, piperazine–H), 2.61 (s, 3H, Thiazol–C_4–CH_3), 4.37 (q, J = 9 Hz, 2H, ester–CH_2), 4.48 (s, 2H, CH_2), 8.10 (brs, 1H, NH). ^{13}C NMR (δ-ppm): 6.9 (CH_3), 13.5 (CH_3), 59.0 (CH_2), 54.6 (piperazine α-C), 57.3 (piperazine β-C), 103.5, 149.3, 172.2 (Thiazole C), 167.2 (CO), 168.1 (CO).

*4.1.8. Ethyl 2-(3-substituted ureido)-4-methylthiazole-5-carboxylates (**9a**,**b**)*

A solution of the thiazole **1** (0.93 g, 5 mmol) and the appropriate isocyanate (6 mmol) in pyridine (10 mL), was heated under reflux for 6–8 h. After cooling to room temperature, the reaction mixture was poured onto 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}): 3370–3265 (NH_2), 1729 (C=O), 1650–1645 (C=O).

*4.1.8.1. Ethyl 2-(3-cyclohexylureido)-4-methylthiazole-5-carboxylate (**9a**).* ^{1}H NMR (δ-ppm): 1.34 (t, J = 9 Hz, 3H, ester–CH_3), 1.48–1.73 (m, 10H, cyclohexyl–H), 2.29 (m, 1H, cyclohexyl–H), 2.57 (s, 3H, Thiazol–C_4–CH_3), 4.29 (q, J = 9 Hz, 2H, ester–CH_2), 8.16 (brs, 2H, 2 NH). ^{13}C NMR (δ-ppm): 6.9 (CH_3), 13.7 (CH_3), 21.6, 27.2, 32.6, 47.4 (cyclohexyl C) 59.1 (CH_2), 103.1, 148.9, 171.8 (Thiazole C), 157.5 (CO), 167.1 (CO).

*4.1.8.2. Ethyl 4-methyl-2-(3-phenylureido)thiazole-5-carboxylate (**9b**).* ^{1}H NMR (δ-ppm): 1.36 (t, J = 9 Hz, 3H, ester–CH_3), 2.59 (s, 3H, Thiazol–C_4–CH_3), 4.26 (q, J = 9 Hz, 2H, ester–CH_2), 6.98–7.47 (m, 5H, Ar–H), 8.2 (brs, 2H, 2 NH). ^{13}C NMR (δ-ppm): 6.8 (CH_3), 13.6 (CH_3), 59.2 (CH_2), 103.6, 149.4, 172.3 (Thiazole C), 120.4, 124.1, 128.6, 138.4 (Ar C), 152.5 (CO), 167.0 (CO).

*4.1.9. 3-(5-(Hydrazinecarbonyl)-1-substituted-4-methylthiazol-2-yl)urea (**10a**,**b**)*

A solution of the appropriate ureido derivative **9** (5 mmol) in ethanol (15 mL) was treated with hydrazine hydrate 99% (0.75 g, 15 mmol) and the reaction mixture was heated under reflux for 4 h. The obtained precipitate, upon cooling, was filtered, washed with cold ethanol, dried and recrystallized. Physicochemical and analytical data are recorded in Table 3. IR (cm^{-1}): 3340–3198 (NH), 1678–1670 (C=O), 1664–1657 (C=O).

*4.1.9.1. 1-Cyclohexyl-3-(5-(hydrazinecarbonyl)-4-methylthiazol-2-yl) urea (**10a**).* ^{1}H NMR (δ-ppm): 1.44–1.71 (m, 10H, cyclohexyl–H), 2.32 (m, 1H, cyclohexyl–H), 2.49 (s, 3H, Thiazol–C_4–CH_3), 6.08 (brs, 1H, NH), 8.15 (brs, 1H, NH), 8.29 (brs, 1H, NH). ^{13}C NMR (δ-ppm): 6.7 (CH_3), 21.5, 27.1, 32.7, 47.2 (cyclohexyl C), 103.5, 148.7, 171.6 (Thiazole C), 157.3 (CO), 167.2 (CO).

*4.1.9.2. 1-(5-(Hydrazinecarbonyl)-4-methylthiazol-2-yl)-3-phenylurea (**10b**).* ^{1}H NMR (δ-ppm): 2.48 (s, 3H, Thiazol–C_4–CH_3), 5.97 (brs, 1H, NH), 7.05–7.72 (m, 5H, Ar–H), 8.06 (brs, 1H, NH), 8.21 (brs, 1H, NH). ^{13}C NMR (δ-ppm): 7.0 (CH_3), 103.4, 149.1, 172.0 (Thiazole C), 120.3, 124.2, 128.8, 138.6 (Ar C), 152.5 (CO), 167.3 (CO).

*4.1.10. Ethyl 2-(3-substituted thioureido)-4-methylthiazole-5-carboxylates (**11a**–**f**)*

To a solution of the thiazole **1** (0.93 g, 5 mmol) in pyridine (10 mL) was added the appropriate isothiocyanate (6 mmol). The reaction mixture was heated under reflux for 5–6 h then allowed to attain room temperature. Working up of the reaction mixture was carried out as described under **9a**,**b**. Physicochemical and analytical data are recorded in Table 3. IR (cm^{-1}): 3470–2935 (NH), 1730–1718 (C=O), 982–958 (NCS).

*4.1.10.1. Ethyl 4-methyl-2-(3-methylthioureido)thiazole-5-carboxylate (**11a**).* ^{1}H NMR (δ-ppm): 1.36 (t, J = 9 Hz, 3H, ester–

CH_3), 2.52 (s, 3H, Thiazol–C_4–CH_3), 3.22 (s, 3H, N–CH_3), 4.27 (q, $J = 9$ Hz, 2H, ester–CH_2), 6.21 (brs, 2H, 2 NH). ^{13}C NMR (δ-ppm): 6.8 (CH_3), 13.7 (CH_3), 34.0 (N–CH_3), 59.1 (CH_2), 103.2, 149.1, 172.0 (Thiazole C), 167.0 (CO), 179.6 (CS).

*4.1.10.2. Ethyl 2-(3-cyclohexylthioureido)-4-methylthiazole-5-carboxylate (**11b**).* ^{1}H NMR (δ-ppm): 1.32 (t, $J = 9$ Hz, 3H, ester–CH_3), 1.52–1.69 (m, 10H, cyclohexyl–H), 2.26 (m, 1H, cyclohexyl–H), 2.52 (s, 3H, Thiazol–C_4–CH_3), 4.29 (q, $J = 9$ Hz, 2H, ester–CH_2), 6.1 (brs, 2H, 2 NH). ^{13}C NMR (δ-ppm): 6.8 (CH_3), 13.6 (CH_3), 22.5, 27.1, 33.5, 52.2 (cyclohexyl C) 59.2 (CH_2), 105.0, 148.9, 172.4 (Thiazole C), 167.3 (CO), 179.6 (CS).

*4.1.10.3. Ethyl 2-(3-benzylthioureido)-4-methylthiazole-5-carboxylate (**11c**).* ^{1}H NMR (δ-ppm): 1.35 (t, $J = 9$ Hz, 3H, ester–CH_3), 2.53 (s, 3H, Thiazol–C_4–CH_3), 2.64 (s, 2H, benzyl–CH_2), 4.34 (q, $J = 9$ Hz, 2H, ester–CH_2), 6.35 (brs, 2H, 2 NH), 7.09–7.36 (m, 5H, Ar–H). ^{13}C NMR (δ-ppm): 6.9 (CH_3), 13.7 (CH_3), 55.8 (CH_2), 59.2 (CH_2), 103.2, 148.8, 171.7 (Thiazole C), 126.5, 127.2, 128.4, 142.6 (Ar C), 167.1 (CO), 180.1 (CS).

*4.1.10.4. Ethyl 4-methyl-2-(3-phenylthioureido)thiazole-5-carboxylate (**11d**).* ^{1}H NMR (δ-ppm): 1.34 (t, $J = 9$ Hz, 3H, ester–CH_3), 2.53 (s, 3H, Thiazol–C_4–CH_3), 4.31 (q, $J = 9$ Hz, 2H, ester–CH_2), 6.29 (brs, 2H, 2 NH), 7.12–7.41 (m, 5H, Ar–H). ^{13}C NMR (δ-ppm): 6.9 (CH_3), 13.6 (CH_3), 59.0 (CH_2), 103.4, 149.1, 172.0 (Thiazole C), 124.3, 125.2, 128.8, 139.6 (Ar C), 167.0 (CO), 179.9 (CS).

*4.1.10.5. Ethyl 4-methyl-2-(3-(p-tolyl)thioureido)thiazole-5-carboxylate (**11e**).* ^{1}H NMR (δ-ppm): 1.35 (t, $J = 9$ Hz, 3H, ester–CH_3), 2.27 (s, 3H, tolyl–CH_3), 2.50 (s, 3H, Thiazol–C_4–CH_3), 4.33 (q, $J = 9$ Hz, 2H, ester–CH_2), 6.45 (brs, 2H, 2NH), 6.98–7.23 (m, 4H, Ar–H). ^{13}C NMR (δ-ppm): 6.7 (CH_3), 13.6 (CH_3), 20.9 (CH_3), 59.1 (CH_2), 104.1, 149.3, 172.1 (Thiazole C), 125.2, 129.5, 133.6, 136.4 (Ar C), 167.0 (CO), 180.3 (CS).

*4.1.10.6. Ethyl 2-(3-(4-fluorophenyl)thioureido)-4-methylthiazole-5-carboxylate **(11f)**.* ^{1}H NMR (δ-ppm): 1.37 (t, $J = 9$ Hz, 3H, ester–CH_3), 2.49 (s, 3H, Thiazol–C_4–CH_3), 4.3 (q, $J = 9$ Hz, 2H, ester–CH_2), 6.32 (brs, 2H, 2NH), 6.97–7.19 (m, 4H, Ar–H). ^{13}C NMR (δ-ppm): 6.9 (CH_3), 13.5 (CH_3), 59.2 (CH_2), 103.3, 149.2, 172.3 (Thiazole C), 115.9, 126.8, 135.1, 158.3 (Ar C), 167.0 (CO), 180.2 (CS).

*4.1.11. 1-(5-(Hydrazinecarbonyl)-4-methylthiazol-2-yl)-3-(substituted)thiourea (**12a–f**)*

To a solution of the appropriate thioureido derivative **11** (5 mmol) in ethanol (15 mL) was added hydrazine hydrate (0.75 g, 15 mmol), and the reaction mixture was heated under reflux for 6 h. The obtained precipitate, upon cooling, was filtered, washed with cold water, dried and recrystallized. Physicochemical and analytical data are recorded in Table 2. IR (cm^{-1}): 3540–2920 (NH), 1670–1665 (C=O), 990–953 (NCS).

*4.1.11.1. 1-(5-(Hydrazinecarbonyl)-4-methylthiazol-2-yl)-3-methylthiourea (**12a**).* ^{1}H NMR (δ-ppm): 2.55 (s, 3H, Thiazol–C_4–CH_3), 3.25 (s, 3H, N–CH_3), 5.58 (brs, 1H, NH), 6.19 (brs, 1H, NH), 8.12 (brs, 1H, NH). ^{13}C NMR (δ-ppm): 7.4 (CH_3), 34.2 (N–CH_3), 104.4, 149.2, 171.9 (Thiazole C), 167.4 (CO), 179.9 (CS).

*4.1.11.2. 1-Cyclohexyl-3-(5-(hydrazinecarbonyl)-4-methylthiazol-2-yl)thiourea (**12b**).* ^{1}H NMR (δ-ppm): 1.49–1.58 (m, 10H, cyclohexyl–H), 2.17 (m, 1H, cyclohexyl–H), 2.59 (s, 3H, Thiazol–C_4–CH_3), 6.16 (brs, 1H, NH), 8.09 (brs, 1H, NH). ^{13}C NMR (δ-ppm): 7.6 (CH_3), 22.3, 27.3, 32.9, 51.2 (cyclohexyl C), 104.6, 148.7, 172.0 (Thiazole C), 167.4 (CO), 179.8 (CS).

*4.1.11.3. 1-Benzyl-3-(5-(hydrazinecarbonyl)-4-methylthiazol-2-yl) thiourea (**12c**).* ^{1}H NMR (δ-ppm): 2.61 (s, 3H, Thiazol–C_4–CH_3), 4.93 (s, 2H, CH_2), 5.54 (brs, 1H, NH), 6.14 (brs, 1H, NH), 7.21–7.78 (m, 5H, Ar–H), 8.06 (brs, 1H, NH). ^{13}C NMR (δ-ppm): 7.5 (CH_3), 55.9 (CH_2), 103.2, 148.8, 171.7 (Thiazole C), 126.5, 127.2, 128.4, 142.6 (Ar C), 167.1 (CO), 180.1 (CS).

*4.1.11.4. 1-(5-(Hydrazinecarbonyl)-4-methylthiazol-2-yl)-3-phenylthiourea (**12d**).* ^{1}H NMR (δ-ppm): 2.48 (s, 3H, Thiazol–C_4–CH_3), 5.66 (brs, 1H, NH), 5.98 (brs, 1H, NH), 7.34–7.84 (m, 5H, Ar–H), 8.10 (brs, 1H, NH). ^{13}C NMR (δ-ppm): 7.5 (CH_3), 103.4, 149.1, 172.0 (Thiazole C), 124.6, 125.8, 128.8, 139.4 (Ar C), 167.2 (CO), 179.8 (CS).

*4.1.11.5. 1-(5-(Hydrazinecarbonyl)-4-methylthiazol-2-yl)-3-(p-tolyl) thiourea (**12e**).* ^{1}H NMR (δ-ppm): 2.32 (s, 3H, tolyl–CH_3), 2.51 (s, 3H, Thiazol–C_4–CH_3), 4.45 (brs, 1H, NH), 5.8 (brs, 1H, NH), 7.11–7.48 (m, 4H, Ar–H), 8.11 (brs, 1H, NH). ^{13}C NMR (δ-ppm): 6.7 (CH_3), 20.9 (CH_3), 103.8, 149.2, 172.0 (Thiazole C), 125.4, 129.7, 133.7, 136.8 (Ar C), 167.6 (CO), 180.1 (CS).

*4.1.11.6. 1-(4-Fluorophenyl)-3-(5-(hydrazinecarbonyl)-4-methylthiazol-2-yl)thiourea (**12f**).* ^{1}H NMR (δ-ppm): 2.45 (s, 3H, Thiazol–C_4–CH_3), 4.05 (brs, 1H, NH), 5.14 (brs, 1H, NH), 7.12–7.62 (m, 4H, Ar–H), 7.99 (brs, 1H, NH). ^{13}C NMR (δ-ppm): 6.9 (CH_3), 103.7, 148.2, 171.7 (Thiazole C), 115.7, 126.6, 135.3, 158.4 (Ar C), 167.5 (CO), 180.0 (CS).

*4.1.12. 2-Amino-4-methyl-N-substituted-thiazole-5-carboxamides (**13a–c**)*

To a solution of the thiazole **1** (0.93 g, 5 mmol) in ethanol (15 mL), the appropriate amine was added and the mixture was heated under reflux for 10–12 h. After being cooled to room temperature, the separated solid was filtered, washed with cold ethanol, dried and recrystallized. Physicochemical and analytical data are recorded in Table 3. IR (cm^{-1}): 3540–2775 (NH, OH), 1685–1645 (C=O).

*4.1.12.1. 2-Amino-N-(4-hydroxyethyl)-4-methylthiazole-5-carboxamide (**13a**).* ^{1}H NMR (δ-ppm): 2.51 (s, 3H, Thiazol–C_4–CH_3), 2.90 (m, 2H, CH_2), 3.46 (m, 2H, CH_2), 3.85 (brs, 1H, OH), 6.13 (brs, 2H, NH_2), 8.16 (brs, 1H, NH). ^{13}C NMR (δ-ppm): 6.5 (CH_3), 103.5, 149.2, 172.2 (Thiazole C), 45.4 (CH_2–N), 64.5 (CH_2–OH), 167.9 (CO).

*4.1.12.2. 2-Amino-N-(4-methoxyphenyl)-4-methylthiazole-5-carboxamide (**13b**).* ^{1}H NMR (δ-ppm): 2.43 (s, 3H, Thiazol–C_4–CH_3), 3.51 (s, 3H, OCH_3), 6.48 (brs, 2H, NH_2), 7.14–7.72 (m, 4H, Ar–H), 8.12 (brs, 1H, NH). ^{13}C NMR (δ-ppm): 6.6 (CH_3), 56.0 (CH_3O), 103.7, 149.0, 172.3 (Thiazole C), 114.3, 121.4, 130.5, 157.5 (Ar C), 165.4 (CO).

*4.1.12.3. 2-Amino-N-(4-fluorophenyl)-4-methylthiazole-5-carboxamide (**13c**).* ^{1}H NMR (δ-ppm): 2.48 (s, 3H, Thiazol–C_4–CH_3), 6.09 (brs, 2H, NH_2), 6.88–7.52 (m, 4H, Ar–H), 8.08 (brs, 1H, NH). ^{13}C NMR (δ-ppm): 6.5 (CH_3), 103.5, 149.3, 172.1 (Thiazole C), 115.6, 122.2, 133.5, 157.7 (Ar C), 165.2 (CO).

4.2. In vitro antibacterial and antifungal screening

4.2.1. Inhibition-zone (IZ) measurements

All the synthesized compounds were evaluated by the agar cup diffusion technique [41] using a 1 mg/mL solution in DMSO. The test organisms utilized were *S. aureus* (ATCC 6538), *B. subtilis* (ATCC 6633) as examples of Gram positive bacteria and *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) as examples of Gram negative bacteria. They were also evaluated for their *in vitro* antifungal potential against *C. albicans* (ATCC 10231) and *A. niger* (recultured)

fungal strains. Each 100 mL of sterile molten agar (at 45 °C) received 1 mL of 6 h-broth culture and then the seeded agar was poured into sterile Petri dishes. Cups (8 mm in diameter) were cut in the agar. Each cup received 0.1 mL of the 1 mg/mL solution of the test compounds. The plates were then incubated at 37 °C for 24 h or, in case of *C. albicans*, for 48 h. A control using DMSO without the test compound was included for each organism. Ampicillin trihydrate and gentamicin sulfate were used as standard antibacterial agents, whereas clotrimazole and amphotericin B were used as antifungal reference drugs. The results were recorded for each tested compound as the average of three measurements of the diameter of inhibition zones (IZ) of bacterial or fungal growth around the discs in mm. The minimum inhibitory concentration (MIC, μg/mL) and minimum bacterial concentration (MBC, μg/mL) measurements were determined for compounds that showed significant growth inhibition zones (≥13 mm) using the two-fold serial dilution method [42].

4.2.2. Minimal inhibitory concentration (MIC) measurement

The minimal inhibitory concentrations (MIC) of the most active compounds were measured using the twofold serial broth dilution method [42]. The test organisms were grown in their suitable broth: 24 h for bacteria and 48 h for fungi at 37 °C. Twofold serial dilutions of solutions of the test compounds were prepared using 200, 100, 50, 25, 12.5 and 6.25 μg/mL. The tubes were then inoculated with the test organisms; each 5 mL received 0.1 mL of the above inoculum and were incubated at 37 °C for 48 h. Then, the tubes were observed for the presence or absence of microbial growth. The MIC values of the prepared compounds are listed in Table 1.

4.2.3. Minimal bactericidal concentration (MBC) measurement

MIC tests were always extended to measure the MBC [43] as follows: A loop-full from the tube not showing visible growth (MIC) was spread over a quarter of Müller–Hinton agar plate. After 18 h of incubation, the plates were examined for growth. Again, the tube containing the lowest concentration of the test compound that failed to yield growth on subculture plates was judged to contain the MBC of that compound for the respective test organism (Table 1).

4.3. Preliminary in vitro anticancer screening

4.3.1. Primary in vitro one-dose assay

Out of the newly synthesized compounds, nine derivatives **4**, **5a**, **9a**,**b**, **10a**,**b**, **12f** and **13a**,**c** 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. 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 [44–46]. 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 (Table 2).

Acknowledgment

The authors are very grateful to the staff members of the Department of Health and Human Services, National Cancer Institute (NCI), Bethesda, Maryland, U.S.A. for carrying out the anticancer screening of the newly synthesized compounds. Extendable thanks are conveyed to the staff of the Genetic Engineering and Biotechnology Research Institute, City for Scientific Research and Technology Applications, Alexandria, Egypt, for their efforts in carrying out the microbiological screening.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.02.027.

References

[1] E. Akbas, I. Berber, European Journal of Medicinal Chemistry 40 (2005) 401–405.
[2] I.R. Ezabadi, C. Camoutsis, P. Zoumpoulakis, A. Geronikaki, M. Sokovic, J. Glamocilija, A. Ciric, Bioorganic & Medicinal Chemistry 16 (2008) 1150–1161.
[3] S. Eckhardt, Current Medicinal Chemistry – Anti-Cancer Agents 2 (2002) 419–439.
[4] T.M. de Aquino, A.P. Liesen, R.E.A. da Silva, V.T. Lima, C.S. Carvalho, A.R. de Faria, J.M. de Araujo, J.G. de Lima, A.J. Alves, E.J.T. de Melo, A.J.S. Goes, Bioorganic & Medicinal Chemistry 16 (2008) 446–456.
[5] T. Kline, H.B. Felise, K.C. Barry, S.R. Jackson, H.V. Nguyen, S.I. Miller, Journal of Medicinal Chemistry 51 (2008) 7065–7074.
[6] M.A. Gouda, M.A. Berghot, Ghada E. Abd El-Ghani, A.M. Khalil, European Journal of Medicinal Chemistry 45 (2010) 1338–1345.
[7] S. Bondock, T. Naser, Y.A. Ammar, European Journal of Medicinal Chemistry 62 (2013) 270–279.
[8] K. Omar, A. Geronikaki, P. Zoumpoulakis, C. Camoutsis, M. Sokovic, A. Ciric, J. Glamocilija, Bioorganic & Medicinal Chemistry 18 (2010) 426–432.
[9] F. Chimenti, B. Bizzarri, A. Bolasco, D. Secci, P. Chimenti, A. Granese, S. Carradori, M. D'Ascenzio, D. Lilli, D. Rivanera, European Journal of Medicinal Chemistry 46 (2011) 378–382.
[10] G.V. Suresh Kumar, Y. Rajendraprasad, B.P. Mallikarjuna, S.M. Chandrashekar, C. Kistayya, European Journal of Medicinal Chemistry 45 (2010) 2063–2074.
[11] P. Makam, R. Kankanala, A. Prakash, T. Kannan, European Journal of Medicinal Chemistry 69 (2013) 564–576.
[12] M.P. Hay, S. Turcotte, J.U. Flanagan, M. Bonnet, D.A. Chan, P.D. Sutphin, P. Nguyen, A.J. Giaccia, W.A. Denny, Journal of Medicinal Chemistry 53 (2010) 787–797.
[13] M.S. Al-Said, M.S. Bashandy, S.H.I. Al-qasoumi, M.M. Ghorab, European Journal of Medicinal Chemistry 46 (2011) 137–141.
[14] R. Romagnoli, P.G. Baraldi, M.K. Salvador, D. Preti, M.A. Tabrizi, A. Brancale, X.-H. Fu, J. Li, S.-Z. Zhang, E. Hamel, R. Bortolozzi, E. Porcù, G. Basso, G. Viola, Journal of Medicinal Chemistry 55 (2012) 5433–5445.
[15] A. Meriç, Z. İncesu, İ. Hatipoğlu, Medicinal Chemistry Research 17 (2008) 30–41.
[16] Yu Luo, Feng Xiao, Shijing Qian, Wei Lu, Bo Yang, European Journal of Medicinal Chemistry 46 (2011) 417–422.
[17] L. Garuti, M. Roberti, A. Pession, E. Leoncini, S. Hrelia, Bioorganic & Medicinal Chemistry Letters 11 (2001) 3147–3149.
[18] Y. Lu, C.-M. Li, Z. Wang, C.R. Ross, J. Chen, J.T. Dalton, W. Li, D.D. Miller, Journal of Medicinal Chemistry 52 (2009) 1701–1711.
[19] R. Cejudo, G. Alzuet, M. Gonzalez-Alvarez, J.L. Garcia-Gimenez, J. Borras, M. Liu-Gonzalez, Journal of Inorganic Biochemistry 100 (2006) 70–79.
[20] S. Chandrappa, H. Chandru, A.C. Sharada, K. Vinaya, C.S.A. Kumar, N.R. Thimmegowda, P. Nagegowda, M.K. Kumar, K.S. Rangappa, Medicinal Chemistry Research 19 (2010) 236–249.
[21] R. Romagnoli, P.G. Baraldi, M.D. Carrion, O. Cruz-Lopez, C.L. Cara, G. Basso, G. Viola, M. Khedr, J. Balzarini, S. Mahboobi, A. Sellmer, A. Brancale, E. Hamel, Journal of Medicinal Chemistry 52 (2009) 5551–5555.
[22] S. Zheng, Q. Zhong, Q. Jiang, M. Mottamal, Q. Zhang, N. Zhu, M.E. Burow, R.A. Worthylake, G. Wang, ACS Medicinal Chemistry Letters 4 (2013) 191–196.
[23] M.F. Earle, R.I. Glazer, Cancer Research 43 (1983) 133–137.
[24] P. Callery, P. Gannett, in: W.O. Foye, T.L. Lemke, D.A. Williams (Eds.), Principles of Medicinal Chemistry, fifth ed., Lippincott Williams and Wilkins, USA, 2002, pp. 938–941.
[25] A.I. Khalaf, N. Anthony, D. Breen, G. Donoghue, S.P. Mackay, F.J. Scott, C.J. Suckling, European Journal of Medicinal Chemistry 46 (2011) 5343–5355.
[26] A. Szilagyi, F. Fenyvesi, O. Majercsik, I.F. Pelyas, I. Bacskay, P. Fehér, J. Varadi, M. Vecsernyés, P. Herczegh, Journal of Medicinal Chemistry 49 (2006) 5626–5630.
[27] J.S. Tokarski, J.A. Newitt, C.Y.J. Chang, J.D. Cheng, M. Wittekind, S.E. Kiefer, K. Kish, F.Y.F. Lee, R. Borzillerri, L.J. Lombardo, D.L. Xie, Y.Q. Zhang, H.E. Klei, Cancer Research 66 (2006) 5790–5797.
[28] H.T.Y. Fahmy, S.A.F. Rostom, A.A. Bekhit, Archiv der Pharmazie – Pharmaceutical and Medicinal Chemistry 335 (2002) 213–222.
[29] A.A. Bekhit, H.T.Y. Fahmy, S.A.F. Rostom, A.M. Baraka, European Journal of Medicinal Chemistry 38 (2003) 27–36.
[30] H.T.Y. Fahmy, S.A.F. Rostom, M.N.S. Saudi, J.K.D. Zjawiony, J. Robins, Archiv der Pharmazie – Pharmaceutical and Medicinal Chemistry 336 (2003) 216–225.
[31] S.A.F. Rostom, Bioorganic & Medicinal Chemistry 14 (2006) 6475–6485.

[32] H.M. Faidallah, M.S. Al-Saadi, S.A.F. Rostom, H.T.Y. Fahmy, Medicinal Chemistry Research 16 (2007) 300–318.
[33] M.S. Al-Saadi, S.A.F. Rostom, H.M. Faidallah, Archiv der Pharmazie – Chemistry in Life Sciences 341 (2008) 181–190.
[34] M.S. Al-Saadi, H.M. Faidallah, S.A.F. Rostom, Archiv der Pharmazie – Chemistry in Life Sciences 341 (2008) 424–434.
[35] S.A.F. Rostom, I.M. El-Ashmawy, H.A. Abd El Razik, M.H. Badr, H.M.A. Ashour, Bioorganic & Medicinal Chemistry 17 (2009) 882–895.
[36] A.A. Bekhit, H.T.Y. Fahmy, S.A.F. Rostom, A.E.A. Bekhit, European Journal of Medicinal Chemistry 45 (2010) 6027–6038.
[37] H.M. Faidallah, K.A. Khan, S.A.F. Rostom, A.M. Asiri, Journal of Enzyme Inhibition and Medicinal Chemistry 28 (2013) 495–508.
[38] H.M. Faidallah, S.A.F. Rostom, K.A. Khan, S.A. Basaif, Journal of Enzyme Inhibition and Medicinal Chemistry 28 (2013) 926–935.
[39] X. Li, X. Lu, M. Xing, X.H. Yang, T.T. Zhao, H.B. Gong, H.L. Zhu, Bioorganic & Medicinal Chemistry Letters 22 (2012) 3589–3593.
[40] H.M. Refat, A.A. Fadda, European Journal of Medicinal Chemistry 70 (2013) 419–426.
[41] S.R. Jain, A. Kar, Planta Medica 20 (1971) 118–123.
[42] A.C. . Scott, in: J.G. Collee, J.P. Duguid, A.G. Fraser, B.P. Marmion (Eds.), Mackie & McCartney Practical Medical Microbiology, thirteenth ed. vol. 2, Churchill Livingstone, New York, 1989, pp. 161–181.
[43] G.L. French, Journal of Antimicrobial Chemotherapy 58 (2006) 1107–1117.
[44] A. Monks, D. Scudiero, P. Skehan, R. Shoemaker, K. Paull, D. Vistica, C. Hose, J. Jangley, P. Cronisie, A. Viagro-Wolff, M. Gray-Goodrich, H. Campell, M. Boyd, Journal of the National Cancer Institute 83 (1991) 757–766.
[45] M.R. Grever, S.A. Schepartz, B.A. Chabner, Seminars in Oncology 19 (1992) 622–638.
[46] M.R. Boyd, K.D. Paull, Drug Development Research 34 (1995) 91–109.
[47] J. Kempson, W.J. Pitts, J. Barbosa, J. Guo, O. Omotoso, A. Watson, K. Stebbins, G.C. Starling, J.H. Dodd, J.C. Barrish, R. Felix, K. Fischer, Bioorganic & Medicinal Chemistry Letters 15 (2005) 1829–1833.
[48] H.I. El-Subbagh, W.A. El-Naggar, F.A. Badria, Medicinal Chemistry Research 3 (1994) 503–516.
[49] H.I. El-Subbagh, A.M. Al-Obaid, European Journal of Medicinal Chemistry 31 (1996) 1017–1021.
[50] S.M. El-Messery, G.S. Hassan, F.A.M. Al-Omary, H.I. El-Subbagh, European Journal of Medicinal Chemistry 54 (2012) 615–625.
[51] G.S. Hassan, S.M. El-Messery, F.A.M. Al-Omary, H.I. El-Subbagh, Bioorganic & Medicinal Chemistry Letters 22 (2012) 6318–6323.
[52] W. Zheng, A. Degterev, E. Hsu, J. Yuan, C. Yuan, Bioorganic & Medicinal Chemistry Letters 18 (2008) 4932–4935.
[53] A. Aliabadi, F. Shamsa, S.N. Ostad, S. Emami, A. Shafiee, J. Davoodi, A. Foroumadi, European Journal of Medicinal Chemistry 45 (2010) 5384–5389.
[54] G.S. Hassan, S.M. El-Messery, F.A.M. Al-Omary, S.T. Al-Rashood, M.I. Shabayek, Y.S. Abulfadl, E.E. Habib, S.M. El-Hallouty, W. Fayad, K.M. Mohamed, B.S. El-Menshaw, H.I. El-Subbagh, European Journal of Medicinal Chemistry 66 (2013) 135–145.
[55] A. Geronikaki, P. Eleftheriou, P. Vicini, I. Alam, A. Dixit, A.K. Saxena, Journal of Medicinal Chemistry 51 (2008) 5221–5228.
[56] N.S. Cutshall, C. O'Day, M. Prezhdo, Bioorganic & Medicinal Chemistry Letters 15 (2005) 3374–3379.