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ORIGINAL PAPER

Synthesis of 2-aminoxazole-5-carbamides and 2-aminothiazole-5-carbamides as potent inhibitors of CML

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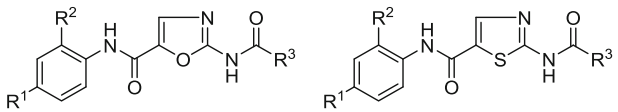
Abstract 2-Aminoxazole-5-carbamide was discovered as novel potent inhibitor against human chronic myeloid leukemia K562 cells; several new corresponding 2-aminothiazole-5-carbamides were also prepared and evaluated the biological activity. The results demonstrated that all of the new compounds exhibited antiproliferative potency on human K562 leukemia cells.

Graphical abstract



Keywords 2-Aminoxazole-5-carbamides · 2-Aminothiazole-5-carbamides · CML · Inhibitor

Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative disorder that is characterized by the presence of a constitutively active tyrosine kinase, Bcr-Abl, which drives the malignant phenotype of leukemic stem cells [1, 2]. Treatment of CML generally relies on Bcr-Abl tyrosine kinase inhibitors. This recognition of the Bcr-Abl gene and corresponding proteins led to the synthesis of small-molecule drugs, which were used to interfere with Bcr-Abl tyrosine kinase activated by competitive binding at the ATP-binding site [3]. Five Bcr-Abl tyrosine kinase inhibitors (TKIs) (imatinib, nilotinib, dasatinib, bosutinib, and ponatinib) are currently approved for the treatment of CML [4]. Within these CML inhibitory categories, imatinib is one of the most well-known molecular drugs for all newly diagnosed CML patients [5, 6]. Dasatinib (Scheme 1), a potent dual inhibitor of Src and Abl kinase, was approved for the oral treatment of CML and Philadelphia chromosome-positive acute lymphoblastic leukemia [2, 7–11]. As a second-generation Bcr-Abl inhibitor, dasatinib has shown significant activity after imatinib failure in clinical trials, but still faces obstacles similar to imatinib, including negligible activity against the frequent Bcr-Abl T315I mutation and modest effects in advanced phases of CML [9]. The emergence of mutations in the Bcr-Abl kinase domain is a common mechanism of TKI resistance; there is a continued interest in developing new agents that have highly potent kinase inhibition profile. A survey of the literature revealed that there had been extensive progress on the preparation of 2-aminothiazole-5-carboxylic acid phenyl amide derivatives [2, 7–11] including dasatinib. To investigate whether the pyrimidin-4-ylamino moiety is critical for activity in this series compounds, acetyl and phenyl acetyl substitution were adopted as alternate scaffolds at the 2-amino group, and some derivatives with the absence of the chloro atom at the phenyl ring moiety were reported as TKIs [2, 8–10]. However, modification on replacement of the thiazole core has not been reported so far. Considering oxazole and thiazole are bioisosteres, we made an effort to design a series of 2-aminoxazole-5-

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carbamide compounds, aimed to observe whether these different heterocyclic compounds favor greater inhibition of cell proliferation and higher induction of cell death in treatment of CML. Herein, a new and efficient method has been developed for the synthesis of 2-aminoxazole-5-carbamides and 2-aminothiazole-5-carbamides, with their in vitro cytostatic effect screened on human chronic myeloid leukemia cell line K562.

Results and discussion

Synthesis

Preparation of the two series compounds is outlined in Scheme 1. (*E*)-3-Ethoxy-*N*-arylpropenamides **3a**–**3c** served as advanced intermediates, synthesized by nucleophilic substitution of appropriate aniline onto (*E*)-3-ethoxyacryloyl chloride (**2**), which was obtained in one pot from ethyl vinyl ether (**1**) and oxalyl chloride by Effenberger addition followed by thermal decarbonylation according to the literature [12]. Reaction of the advanced enones **3a**–**3c** with *N*-bromosuccinimide (NBS) followed by a coupling of urea or thiourea installs the oxazole skeleton in **4a**–**4c** or thiazole ring in **5a**–**5c** [9, 10]. The final step was a nucleophilic displacement of acetic anhydride or different benzoyl chloride onto **4a**–**4c** or **5a**–**5c** to yield the corresponding target **6a**–**6g** and **7a**–**7h**, respectively.

Anti-CML activity

All newly synthesized compounds were evaluated on K562 cell in vitro for anti-CML activity, in comparison with marketed drug dasatinib and imatinib used as references, and the assays are summarized in Table 1. They are expressed as IC_{50}, a concentration at which the compounds inhibit the cell proliferation for 50 %. The results displayed an encouraging spectrum of cell inhibitory activity, especially five compounds **4b**, **6c**, **7a**, **7f**, **7h**, which were more potent than the other analogs including imatinib.

In a direct comparison of 2-aminoxazole-5-carbamides (**6a**, **6c**, **6d**, **6e**, **6g**) with their counterparts 2-aminothiazole-5-carbamides (**7a**, **7c**, **7d**, **7e**, **7f**), similar potent and broad spectrum cellular activity were observed; it might be explained that whether thiazole or oxazole scaffold in the heterocyclic compounds is crucial for the potency; its electrostatic field effect in the interaction of molecular with Bcr-Abl has no significant role on the whole set of the interactions. As part of a systematic preliminary structure–activity relationship (SAR) approach, we first investigated the importance of the carboxanilide side chain on the 5-position of oxazole and thiazole ring, and found that inhibitory activity was weakly affected by substitution at this position.

Our next effort focused on developing the SAR with respect to the substitution pattern on the 2-aminooxazole skeleton. To our surprise, when benzamide or *para*-methylbenzamide attended to 5-position of the oxazole ring, improvement in the cellular activity profile was observed when 2-NH_2 group was *N*-acylated by benzoyl substituent (**6d**, **6c**) prior to acetyl group (**6b**, **6g**, and **6a**, **6f**, respectively), it is hypothesized that the specific interaction of aromatic ring with Abl is responsible for favorable activity of this series compounds. To evaluate electron withdrawing effect on the potency, compound **7h** was synthesized and identified as the most potent inhibitor versus the K562 cell line in this series, almost the equivalent inhibition percentage compared with dasatinib, this result implied that an

Scheme 1

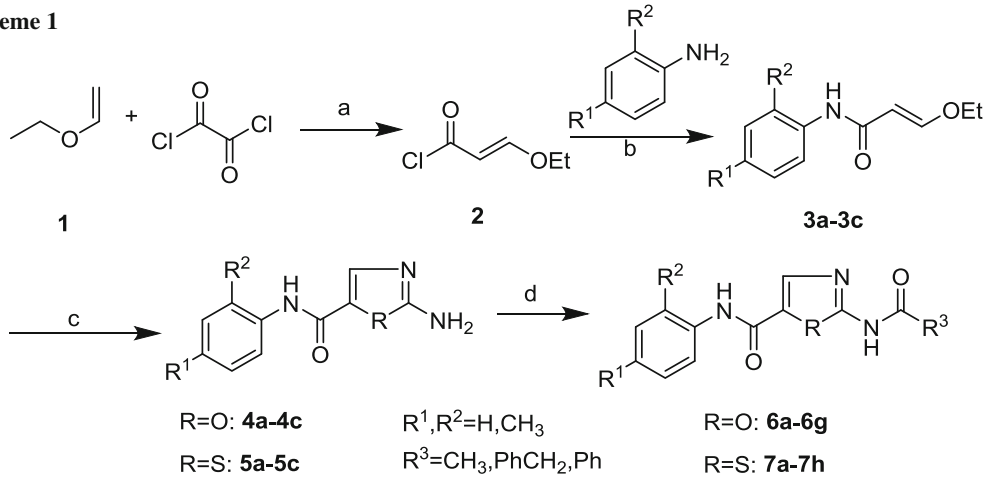
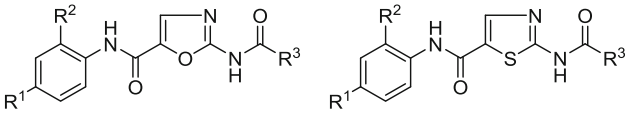


Table 1 The inhibitory activity against K562 cell of novel compounds



6a-6g **7a-7h**

	R^1	R^2	R^3	IC_{50}/μM
4a	CH_3	H		0.174 ± 0.046
4b	H	CH_3		0.090 ± 0.002
4c	H	H		0.185 ± 0.033
6a	CH_3	H	CH_3	0.172 ± 0.027
6b	H	H	CH_3	0.159 ± 0.034
6c	CH_3	H	Ph	0.087 ± 0.003
6d	H	H	Ph	0.125 ± 0.016
6e	H	CH_3	Ph	0.109 ± 0.035
6f	CH_3	H	$PhCH_2$	0.149 ± 0.028
6g	H	H	$PhCH_2$	0.134 ± 0.037
7a	CH_3	H	CH_3	0.080 ± 0.004
7b	H	CH_3	CH_3	0.166 ± 0.051
7c	H	H	CH_3	0.222 ± 0.062
7d	CH_3	H	Ph	0.121 ± 0.033
7e	H	H	Ph	0.129 ± 0.041
7f	H	CH_3	Ph	0.093 ± 0.006
7g	H	H	$PhCH_2$	0.111 ± 0.016
7h	H	CH_3	3-NO_2–Ph	0.060 ± 0.003
Dasatinib				0.050 ± 0.002
Imatinib				0.112 ± 0.045

improvement of the biological profile could be obtained by the introduction of electron withdrawing substituent group (e.g., NO_2) to the benzoyl ring.

In summary, we identified a novel oxazole lead as a CML inhibitor; further development of the SAR, and optimization of the cell and in vivo activity of this series will be reported in due course.

Experimental

^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker AV 600 MHz spectrometer. Chemical shifts were expressed in ppm downfield from TMS, which was used as an internal standard. Mass spectra were obtained on an Agilent MS/5975 mass spectrometer. IR spectra (KBr) were recorded on a JASCO FT/IR-8400 instrument. Elemental analysis was performed on a Carloerba 1106 instrument and the results of elemental analysis for C, H, N, S were within 0.4 % of the theoretical values. All the reactions were monitored by TLC on pre-coated silica gel G plates at 254 nm under a UV lamp. All chemicals and solvents were reagent grade purified by standard methods prior to use.

*(E)-3-Ethoxy-N-arylpropenamides **3a–3c***

The preparation of (*E*)-3-ethoxyacryloyl chloride (**2**) was according to the literature [13]. To a solution of aniline (28 mmol) and 3.2 cm^3 pyrimidine (40 mmol) in 40 cm^3 THF was added 3.6 cm^3 (*E*)-3-ethoxyacryloyl chloride (30 mmol) dropwise at 0 °C, after stirred for another 2 h at room temperature, 7.0 cm^3 HCl (1 mol/cm^3) was added slowly, the resultant was concentrated in vacuo, the crude product was crystallized from toluene to afford **3a–3c**.

(E)-3-Ethoxy-N-(4-methylphenyl)acryamide
(**3a**, $C_{12}H_{15}NO_2$)
White powder, yield 5.0 g (87.0 %); m.p.: 148–150 °C; ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 9.29$ (s, 1H, –NH–CO–), 7.40–7.46 (m, 2H, Ar–H), 7.32 (d, $J = 12.4$ Hz, 1H, –CO–CH=), 7.18–7.23 (m, 2H, Ar–H), 5.58 (d, $J = 12.4$ Hz, 1H, –O–CH=), 3.94 (q, $J = 7.0$ Hz, 2H, –CH_2–), 2.16 (s, 3H, –CH_3), 1.27 (t, $J = 7.0$ Hz, 3H, –CH_3)

ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 19.11 (CH_3), 19.34 (CH_3), 41.44 (CH_2), 66.76 (O–CH=), 75.87 (=CH), 119.23 (2), 124.58, 129.50 (2), 135.66, 152.36 (C=O, carboxamide) ppm; IR (KBr): $\bar{\nu}$ = 3,257, 1,662, 1,508, 1,292, 1,161 cm^{-1}; MS (70 eV): *m*/*z* = 205 (M^+).

(E)-3-Ethoxy-N-phenylacrylamide (**3b**, $C_{11}H_{13}NO_2$)
Yellow powder, yield 4.8 g (89.4 %); m.p.: 79–81 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 9.00 (s, 1H, –NH–CO–), 7.50 (m, 1H, Ar–H), 7.32–7.29 (m, 2H), 7.18–7.23 (m, 2H, Ar–H), 5.58 (d, *J* = 12.4 Hz, 1H, –O–CH=), 4.00 (q, *J* = 7.0 Hz, 2H, –CH_2–), 1.22 (t, *J* = 7.0 Hz, 3H, –CH_3) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 19.35 (CH_3), 41.46 (CH_2), 66.78 (O–CH=), 73.56 (=CH), 119.59 (2), 129.36 (2), 135.67, 137.82, 150.56 (C=O, carboxamide) ppm; IR (KBr): $\bar{\nu}$ = 3,261, 1,660, 1,621, 1,509, 1,408, 1,357, 1,292, 1,244, 1,161 cm^{-1}; MS (70 eV): *m*/*z* = 191 (M^+).

*(E)-3-Ethoxy-N-(2-methylphenyl)acrylamide (**3c**)*
Yellow powder, yield 4.9 g (85.4 %); m.p.: 92–94 °C (Ref. [9, 10] 92–96 °C)

*2-Amino-N-aryloxazole-5-carboxamides **4a–4c***
To a solution of **3a–3c** (25 mmol) in 40 cm^3 H_2O and 40 cm^3 1,4-dioxane was added 4.4 g NBS (25 mmol) at 0 °C while kept stirring for 1 h, after warmed to room temperature and stirred for another 2 h, 4.4 g urea (25 mmol) was added, the mixture was allowed to 70 °C and stirred for 2 h, the pH of the resultant was adjusted to 8–9 by ammonia and then concentrated in vacuo. The crude product was crystallized from toluene, affording the counterparts **4a–4c**.

2-Amino-N-(4-methylphenyl)-5-oxazolecarboxamide
(**4a**, $C_{11}H_{11}N_3O_2$)
Yellow solid, yield 2.5 g (45.7 %); m.p.: 271–272 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 9.70 (s, 1H, –NH–CO–), 7.61 (s, 1H, –C=CH–N=), 7.53 (d, *J* = 8.4 Hz, 2H, Ar–H), 7.30 (s, 2H, –NH_2), 7.12 (d, *J* = 8.4 Hz, 2H, Ar–H), 2.26 (s, –CH_3) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 20.96 (CH_3), 120.46 (2), 129.53 (2), 132.10, 133.30, 136.18, 138.55, 155.85 (C=O, carboxamide), 163.45 (O–C=N, oxazole) ppm; IR (KBr): $\bar{\nu}$ = 3,304, 3,103, 1,663, 1,633, 1,607, 1,574, 1,529, 1,173 cm^{-1}; MS (70 eV): *m*/*z* = 217 (M^+).

2-Amino-N-(2-methylphenyl)-5-oxazolecarboxamide
(**4b**, $C_{11}H_{11}N_3O_2$)
Yellow powder, yield 2.4 g (43.7 %); m.p.: 230–232 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 9.31 (s, 1H, –NH–CO–), 7.56 (s, 1H, –C=CH–N=), 7.32 (d, *J* = 7.8 Hz, 1H, Ar–H), 7.26 (s, 2H, NH_2), 7.24 (d, *J* = 7.8 Hz, 1H, Ar–H), 7.19 (t, *J* = 7.2 Hz, 1H, Ar–H), 7.13 (t, *J* = 7.2 Hz, 1H, Ar–H), 2.20 (s, 3H, –CH_3) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 20.86 (CH_3), 120.49, 120.56, 129.50, 129.60, 132.11, 133.35, 136.19, 138.65, 155.87 (C=O, carboxamide), 163.45 (O–C=N, oxazole) ppm; IR (KBr): $\bar{\nu}$ = 3,404, 3,304, 3,067, 2,978, 1,641, 1,614, 1,483, 1,290, 1,178 cm^{-1}; MS (70 eV): *m*/*z* = 217 (M^+).

2-Amino-N-phenyl-5-oxazolecarboxamide
(**4c**, $C_{10}H_9N_3O_2$)
Yellow powder, yield 3.5 g (68.7 %); m.p.: 244–245 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 9.76 (s, 1H, –NH–CO–), 7.66 (d, *J* = 7.2 Hz, 1H, Ar–H), 7.64 (m, 2H, Ar–H), 7.31 (t, *J* = 7.8 Hz, 2H, Ar–H), 7.30 (s, 2H, NH_2), 7.05 (t, *J* = 7.2 Hz, 1H, Ar–H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 120.47 (2), 129.55 (2), 132.10, 133.36, 136.18, 138.55, 155.74 (C=O, carboxamide), 163.21 (O–C=N, oxazole) ppm; IR (KBr): $\bar{\nu}$ = 3,306, 3,105, 1,662, 1,634, 1,612, 1,574, 1,489, 1,283, 1,173 cm^{-1}; MS (70 eV): *m*/*z* = 203 (M^+).

*General procedure for preparation of **6a–6g***

To a solution of **4a–4c** (2.15 mmol) and 0.5 cm^3 Et_3N in 10 cm^3 THF was added benzoyl chloride or acetic anhydride (2.46 mmol) dropwise at 0 °C and stirred for 1 h, then the mixture was warmed to room temperature and stirred for another 2 h, the resultant was concentrated in vacuo. The resulting mixture was partitioned between ethyl acetate and water, and the organic layer was washed with 1 N HCl, 5 % $NaHCO_3$, and brine, and dried over $MgSO_4$. After removal of the solvent under reduced pressure, the residue was purified by silica gel chromatography and then recrystallized from toluene, affording the counterparts **6a–6g**.

2-Acetylamino-N-(4-methylphenyl)-5-oxazolecarboxamide
(**6a**, $C_{13}H_{13}N_3O_3$)
White powder, yield 0.34 g (61.5 %); m.p.: 278–280 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 11.56 (s, 1H, –NH–CO–), 10.10 (s, 1H, –NH–CO–), 7.88 (s, 1H, –C=CH–N=), 7.56 (d, *J* = 7.2 Hz, 2H, Ar–H), 7.16 (d, *J* = 6.6 Hz, 2H, Ar–H), 2.27 (s, 3H, –CH_3), 2.14 (s, 3H, –CH_3) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 20.97 (CH_3), 23.01 (CH_3), 120.79 (2), 128.19, 129.58 (2), 133.20, 136.20, 140.75, 159.84 (C=O, carboxamide), 163.51 (O–C=N, oxazole), 169.45 (C=O, acetamido) ppm; IR (KBr): $\bar{\nu}$ = 3,330, 3,169, 1,688, 1,651, 1,590, 1,288 cm^{-1}; MS (70 eV): *m*/*z* = 259 (M^+).

2-Acetylamino-N-phenyl-5-oxazolecarboxamide
(**6b**, $C_{12}H_{11}N_3O_3$)
White powder, yield 0.40 g (75.4 %); m.p.: 244–246 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 11.58 (s, 1H, –NH–CO–), 10.17 (s, 1H, –NH–CO–), 7.91 (s, 1H, –C=CH–N=), 7.67 (d, *J* = 7.2 Hz, 2H, Ar–H), 7.36 (t, *J* = 7.8 Hz, 2H,

Ar–H), 7.11 (t, $J = 7.2$ Hz, 1H, Ar–H), 2.15 (s, 3H, –CH_3) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): $\delta = 23.80$ (CH_3), 120.74 (2), 128.22, 129.59 (2), 133.26, 136.24, 140.75, 159.89 (C=O, carboxamide), 163.50 (O–C=N, oxazole), 169.40 (C=O, acetamido) ppm; IR (KBr): $\bar{\nu} = 3{,}328$, 3,149, 1,668, 1,650, 1,589, 1,330 cm^{-1}; MS (70 eV): $m/z = 245$ (M^+).

2-Benzoylamino-N-(4-methylphenyl)-5-oxazolecarboxamide (**6c**, $C_{18}H_{15}N_3O_3$)
White powder, yield 0.45 g (65.5 %); m.p.: 261–263 °C; ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 11.94$ (s, 1H, –NH–CO–), 10.17 (s, 1H, –NH–CO–), 8.01 (s, 2H, Ar–H), 7.97 (s, 1H, –C=CH–N=), 7.65 (s, 1H, Ar–H), 7.56 (m, 4H, Ar–H), 7.17 (d, $J = 5.4$ Hz, 2H, Ar–H), 2.28 (s, 3H, –CH_3) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): $\delta = 23.40$ (CH_3), 120.16 (2), 124.41, 127.30, 128.91 (2), 129.39 (2), 129.89 (2), 131.41, 135.21, 138.89, 141.46, 155.12 (C=O, carboxamide), 155.35 (C=O, carboxamide), 163.87 (O–C=N, oxazole) ppm; IR (KBr): $\bar{\nu} = 3{,}373$, 3,007, 1,690, 1,612, 1,539, 1,493, 1,259, 1,151 cm^{-1}; MS (70 eV): $m/z = 321$ (M^+).

2-Benzoylamino-N-phenyl-5-oxazolecarboxamide (**6d**, $C_{17}H_{13}N_3O_3$)
White powder, yield 0.34 g (51.2 %); m.p.: 250–252 °C; ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 11.96$ (s, 1H, –NH–CO–), 10.25 (s, 1H, –NH–CO–), 8.05 (s, 1H, Ar–H), 8.00 (s, 1H, –C=CH–N=), 7.71–7.22 (m, 3H, Ar–H), 7.66 (t, 1H, $J = 7.2$ Hz, Ar–H), 7.56 (t, $J = 7.2$ Hz, 2H, Ar–H), 7.37 (t, $J = 7.2$ Hz, 2H, Ar–H), 7.12 (t, $J = 7.8$ Hz, 1H, Ar–H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): $\delta = 120.09$ (2), 124.42, 127.37, 128.90 (2), 129.27 (2), 129.88 (2), 131.41, 135.21, 138.79, 141.40, 155.11 (C=O, carboxamide), 155.30 (C=O, carboxamide), 163.96 (O–C=N, oxazole) ppm; IR (KBr): $\bar{\nu} = 3{,}387$, 1,709, 1,676, 1,622, 1,499, 1,267, 1,148 cm^{-1}; MS (70 eV): $m/z = 307$ (M^+).

2-Benzoylamino-N-(2-methylphenyl)-5-oxazolecarboxamide (**6e**, $C_{18}H_{15}N_3O_3$)
White powder, yield 0.42 g (61.5 %); m.p.: 227–229 °C; ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 12.93$ (s, 1H, –NH–CO–), 9.90 (s, 1H, –NH–CO–), 8.01 (d, $J = 7.2$ Hz, 2H, Ar–H), 7.94 (s, 1H, –C=CH–N=), 7.65 (t, $J = 7.2$ Hz, 1H, Ar–H), 7.55 (t, $J = 7.2$ Hz, 2H, Ar–H), 7.34 (d, $J = 7.2$ Hz, 1H, Ar–H), 7.28 (d, $J = 7.2$ Hz, 1H, Ar–H), 7.23 (t, $J = 7.2$ Hz, 1H, Ar–H), 7.19 (t, $J = 7.2$ Hz, 1H, Ar–H), 2.26 (s, 3H, –CH_3) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): $\delta = 23.34$ (CH_3), 120.16, 124.42, 127.24, 128.90 (2), 129.36 (2), 129.86, 129.89, 131.41, 132.10, 135.22, 138.79, 141.46, 155.10 (C=O, carboxamide), 155.35 (C=O, carboxamide), 163.82 (O–C=N, oxazole) ppm; IR (KBr): $\bar{\nu} = 3{,}423$, 3,124, 1,713, 1,684, 1,624, 1,456, 1,271, 1,153 cm^{-1}; MS (70 eV): $m/z = 321$ (M^+).

2-Phenylacetamino-N-(4-methylphenyl)-5-oxazolecarboxamide (**6f**, $C_{19}H_{17}N_3O_3$)
White powder, yield 0.29 g (39.6 %); m.p.: 245–247 °C; ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 11.83$ (s, 1H, –NH–CO–), 10.10 (s, 1H, –NH–CO–), 7.88 (s, 1H, –C=CH–N=), 7.56 (d, $J = 7.8$ Hz, 2H, Ar–H), 7.34–7.40 (m, 2H, Ar–H), 7.28–7.33 (m, 3H, Ar–H), 7.12 (d, $J = 8.4$ Hz, 2H, Ar–H), 3.75 (s, 2H, –CO–CH_2–), 2.50 (s, 3H, –CH_3) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): $\delta = 20.99$ (CH_3), 42.91 (CH_2), 120.77 (2), 127.33, 128.89 (2), 129.53 (2), 129.85 (2), 131.15, 133.30, 136.78, 138.55, 141.52, 155.20 (C=O, carboxamide), 155.85 (C=O, carboxamide), 163.46 (O–C=N, oxazole) ppm; IR (KBr): $\bar{\nu} = 3{,}340$, 3,137, 3,059, 2,922, 1,688, 1,645, 1,600, 1,487, 1,300, 1,193 cm^{-1}; MS (70 eV): $m/z = 335$ (M^+).

2-Phenylacetamino-N-phenyl-5-oxazolecarboxamide (**6g**, $C_{18}H_{15}N_3O_3$)
White powder, yield 0.35 g (50.2 %); m.p.: 239–241 °C; ^{1}H NMR (600 MHz, DMSO-d_6) : $\delta = 11.85$ (s, 1H, –NH–CO–), 10.18 (s, 1H, –NH–CO–), 7.93 (s, 1H, –C=CH–N=), 7.60–7.67 (m, $J = 7.8$ Hz, 3H, Ar–H), 7.34–7.44 (m, 4H, Ar–H), 7.25–7.29 (m, 2H, Ar–H), 7.11 (t, $J = 7.2$ Hz, 1H, Ar–H), 3.76 (s, 2H, –CO–CH_2–) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): $\delta = 42.91$ (CH_2), 120.79 (2), 127.33, 128.88 (2), 129.43 (2), 129.85 (2), 131.15, 133.30, 136.78, 138.55, 141.52, 155.20 (C=O, carboxamide), 155.85 (C=O, carboxamide), 163.46 (O–C=N, oxazole) ppm; IR (KBr): $\bar{\nu} = 3{,}322$, 3,060, 2,917, 1,681, 1,632, 1,600, 1,487, 1,304, 1,188 cm^{-1}; MS (70 eV): $m/z = 321$ (M^+).

2-Amino-N-arylthiazole-5-carboxamides ***5a–5c***
To a solution of **3a–3c** (25 mmol) in 40 cm^3 H_2O and 40 cm^3 1,4-dioxane was added 4.4 g NBS (25 mmol) at 0 °C, while kept stirring for 1 h, after warmed to room temperature and stirred for another 2 h, 4.4 g thiourea (25 mmol) was added, the mixture was allowed to 70 °C and stirred for 2 h, the pH of the resultant was adjusted to 8–9 by ammonia and then concentrated in vacuo. The crude product was crystallized from toluene, affording the counterparts **5a–5c**.

2-Amino-N-(4-methylphenyl)-5-thiazolecarboxamide (**5a**, $C_{11}H_{11}N_2OS$)
Yellow powder, yield 5.1 g (87.6 %); m.p.: 240–241 °C; ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 9.77$ (s, 1H, –NH–CO–), 7.87 (s, 1H, –C=CH–N=), 7.60 (s, 2H, –NH_2), 7.52 (d, $J = 8.4$ Hz, 2H, Ar–H), 7.11 (d, $J = 7.8$ Hz, 2H, Ar–H), 2.25 (s, 3H, –CH_3) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): $\delta = 21.16$ (CH_3), 120.55 (2), 129.63 (2), 132.10, 133.30, 136.18, 138.55, 155.75 (C=O, carboxamide), 161.26 (S–C=N, thiazole) ppm; IR (KBr): $\bar{\nu} = 3{,}429$,

3,271, 3,063, 2,866, 1,626, 1,603, 1,531, 1,485, 1,302 cm^{-1}; MS (70 eV): $m/z = 233$ (M^+).

2-Amino-N-(2-methylphenyl)-5-thiazolecarboxamide (**5b**)
Yellow powder, yield 3.7 g (63.5 %); m.p.: 226–228 °C (Ref. [9, 10] 226–230 °C).

2-Amino-N-phenyl-5-thiazolecarboxamide (**5c**, $C_{10}H_9N_3OS$)
Yellow powder, yield 4.4 g (79.5 %); m.p.: 238–240 °C; ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 9.83$ (s, 1H, –NH–CO–), 7.89 (s, 1H, –C=CH–N=), 7.64 (d, $J = 7.8$ Hz, 2H, Ar–H), 7.62 (s, 2H, $–NH_2$), 7.31 (t, $J = 7.8$ Hz, 2H, Ar–H), 7.05 (t, $J = 7.2$ Hz, 1H, Ar–H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): $\delta = 120.42$ (2), 129.53 (2), 132.10, 133.30, 136.18, 138.50, 155.55 (C=O, carboxamide), 161.16 (S–C=N, thiazole) ppm; IR (KBr): $\bar{\nu} = 3{,}275$, 3,092, 1,628, 1,600, 1,549, 1,531, 1,483, 1,273 cm^{-1}; MS (70 eV): $m/z = 219$ (M^+).

*General procedure for preparation of **7a–7 h***

To a solution of **5a**–**5c** (2.15 mmol) and 0.5 cm^3 Et_3N in THF was added benzoyl chloride or acetic anhydride (2.46 mmol) dropwise at 0 °C and stirred for 1 h, then the mixture was warmed to room temperature and stirred for another 2 h, the resultant was concentrated in vacuo. The resulting mixture was partitioned between ethylacetate and water, and the organic layer was washed with 1 N HCI, 5 % $NaHCO_3$, and brine, and dried over $MgSO_4$. After removal of the solvent under reduced pressure, the residue was purified by silica gel chromatography, and then re-crystallized from toluene, affording the desired products **7a**–**7h**.

2-Acetylamino-N-(4-methylphenyl)-5-thiazolecarboxamide (**7a**, $C_{13}H_{13}N_3O_2S$)
White powder, yield 0.27 g (45.4 %); m.p.: 287–288 °C; ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 12.27$ (s, 1H, –NH–CO–), 10.10 (s, 1H, –NH–CO–), 8.28 (s, 1H, –C=CH–N=), 7.57 (d, $J = 4.2$ Hz, 2H, Ar–H), 7.15 (d, $J = 4.2$ Hz, 2H, Ar–H), 2.28 (s, 3H, $–CH_3$), 2.19 (s, 3H, $–CH_3$) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): $\delta = 21.07$ (CH_3), 23.0 (CH_3), 120.81 (2), 128.19, 129.61 (2), 133.20, 136.21, 140.75, 159.84 (C=O, carboxamide), 161.51 (S–C=N, thiazole), 169.44 (C=O, acetamido) ppm; IR (KBr): $\bar{\nu} = 3{,}329$, 1,674, 1,657, 1,593, 1,279 cm^{-1}; MS (70 eV): $m/z = 275$ (M^+).

2-Acetylamino-N-phenyl-5-thiazolecarboxamide (**7b**, $C_{12}H_{11}N_3O_2S$)
White powder, yield 0.40 g (70.4 %); m.p.: 273–275 °C; ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 11.58$ (s, 1H, –NH–CO–), 10.17 (s, 1H, –NH–CO–), 7.99 (s, 1H, –C=CH–N=), 7.67 (d, $J = 7.2$ Hz, 2H, Ar–H), 7.36 (t, $J = 7.8$ Hz, 2H, Ar–H), 7.11 (t, $J = 7.3$ Hz, 1H, Ar–H), 2.15 (s, 3H, $–CH_3$) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): $\delta = 23.08$ (CH_3), 120.77 (2), 128.19, 129.59 (2), 133.20, 136.21, 140.72, 159.85 (C=O, carboxamide), 161.52 (S–C=N, thiazole), 169.39 (C=O, acetamido) ppm; IR (KBr): $\bar{\nu} = 3{,}269$, 1,679, 1,656, 1,589, 1,283 cm^{-1}; MS (70 eV): $m/z = 261$ (M^+).

2-Acetylamino-N-(2-methylphenyl)-5-thiazolecarboxamide (**7c**, $C_{13}H_{13}N_3O_2S$)
White powder, yield 0.33 g (56.7 %); m.p.: 205–207 °C; ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 11.58$ (s, 1H, –NH–CO–), 10.17 (s, 1H, –NH–CO–), 7.91 (s, 1H, –C=CH–N=), 7.68 (d, $J = 7.2$ Hz, 1H, Ar–H), 7.36 (t, $J = 7.8$ Hz, 2H, Ar–H), 7.13 (t, $J = 7.2$ Hz, 1H, Ar–H), 2.28 (s, 3H, $–CH_3$), 2.15 (s, 3H, $–CH_3$) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): $\delta = 20.92$ (CH_3), 23.01 (CH_3), 120.79, 121.80, 128.19, 129.48 129.61, 133.26, 136.20, 140.76, 159.85 (C=O, carboxamide), 161.49 (S–C=N, thiazole), 169.33 (C=O, acetamido) ppm; IR (KBr): $\bar{\nu} = 3{,}155$, 1,668, 1,597, 1,281 cm^{-1}; MS (70 eV): $m/z = 275$ (M^+).

2-Benzoylamino-N-(4-methylphenyl)-5-thiazolecarboxamide (**7d**, $C_{18}H_{15}N_3O_2S$)
White powder, yield 0.47 g (66.1 %); m.p.: 283–285 °C; ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 12.93$ (s, 1H, –NH–CO–), 10.17 (s, 1H, –NH–CO–), 8.01 (s, 1H, –C=CH–N=), 8.13 (d, $J = 7.2$ Hz, 2H, Ar–H), 7.67 (t, $J = 7.2$ Hz, 1H, Ar–H), 7.61 (d, $J = 8.4$ Hz, 2H, Ar–H), 7.57 (t, $J = 7.8$ Hz, 2H, Ar–H), 7.17 (d, $J = 8.4$ Hz, 2H, Ar–H), 2.29 (s, 3H, $–CH_3$) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): $\delta = 23.44$ (CH_3), 120.15 (2), 124.42, 127.34, 128.90 (2), 129.30 (2), 129.88 (2), 131.40, 135.22, 138.79, 141.43, 155.12 (C=O, carboxamide), 155.35 (C=O, carboxamide), 161.51 (S–C=N, thiazole) ppm; IR (KBr): $\bar{\nu} = 3{,}136$, 1,676, 1,634, 1,514, 1,298, 1,182 cm^{-1}; MS (70 eV): $m/z = 337$ (M^+).

2-Benzoylamino-N-phenyl-5-thiazolecarboxamide (**7e**, $C_{17}H_{13}N_3O_2S$)
White powder, yield 0.39 g (53.6 %); m.p.: 232–235 °C; ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 11.96$ (s, 1H, –NH–CO–), 10.25 (s, 1H, –NH–CO–), 8.02 (s, 1H, –C=CH–N=), 7.97–8.00 (m, 2H, Ar–H), 7.71 (d, $J = 7.8$ Hz, 1H, Ar–H), 7.66–7.69 (m, $J = 6.6$ Hz, 2H, Ar–H), 7.56 (t, $J = 7.8$ Hz, 2H, Ar–H), 7.37 (t, $J = 7.8$ Hz, 2H, Ar–H), 7.12 (t, $J = 7.2$ Hz, 1H, Ar–H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): $\delta = 120.10$ (2), 124.42, 127.34, 128.96 (2), 129.27 (2), 129.79 (2), 131.41, 135.23, 138.79, 141.43, 155.12 (C=O, carboxamide), 155.35 (C=O, carboxamide), 161.01 (S–C=N, thiazole) ppm; IR (KBr): $\bar{\nu} = 3{,}238$,

3,062, 2,930, 1,682, 1,634, 1,595, 1,515, 1,489, 1,267, 1,184 cm^{-1}; MS (70 eV): $m/z = 323$ (M^+).

2-Benzoylamino-N-(2-methylphenyl)-5-thiazolecarboxamide (**7f**, $C_{18}H_{15}N_3O_2S$)
White powder, yield 0.47 g (62.4 %); m.p.: 237–239 °C; ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 12.93$ (s, 1H, –NH–CO–), 9.90 (s, 1H, –NH–CO–), 8.13 (d, $J = 7.8$ Hz, 2H, Ar–H), 8.06 (s, 1H, –C=CH–N=), 7.67 (t, $J = 7.8$ Hz, 1H, Ar–H), 7.57 (t, $J = 7.8$ Hz, 2H, Ar–H), 7.34 (d, $J = 7.8$ Hz, 1H, Ar–H), 7.29 (d, $J = 7.2$ Hz, 1H, Ar–H), 7.23 (t, $J = 7.2$ Hz, 1H, Ar–H), 7.18 (t, $J = 7.2$ Hz, 1H, Ar–H), 2.26 (s, 3H, –CH_3) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): $\delta = 23.49$ (CH_3), 120.13, 120.27, 124.42, 127.34, 128.90 (2), 129.27, 129.30, 129.80 (2), 131.40, 135.22, 138.79, 141.43, 155.36 (C=O, carboxamide), 155.35 (C=O, carboxamide), 161.40 (S–C=N, thiazole) ppm; IR (KBr): $\bar{\nu} = 3{,}158$, 3,061, 2,916, 1,680, 1,636, 1,605, 1,490, 1,267 cm^{-1}; MS (70 eV): $m/z = 337$ (M^+).

2-Phenylacetamino-N-(4-methylphenyl)-5–thiazolecarboxamide (**7g**, $C_{19}H_{17}N_3O_2S$)
White powder, yield 0.50 g (70.1 %); m.p.: 224–226 °C; ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 12.63$ (s, 1H, –NH–CO–), 10.11 (s, 1H, –NH–CO–), 8.09 (s, 1H, –C=CH–N=), 7.56 (d, $J = 7.8$ Hz, 2H, Ar–H), 7.34 (m, 4H, Ar–H), 7.27 (m, 1H, Ar–H), 7.14 (d, $J = 7.8$ Hz, 2H, Ar–H), 3.82 (s, 2H, –CO–CH_2), 2.35 (s, 3H, –CH_3) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): $\delta = 20.96$ (CH_3), 42.91 (CH_2), 120.77 (2), 127.33, 128.88 (2), 129.53 (2), 129.87 (2), 131.15, 133.30, 136.78, 138.55, 141.52, 155.20 (C=O, carboxamide), 155.85 (C=O, carboxamide), 161.46 (S–C=N, thiazole) ppm; IR (KBr): $\bar{\nu} = 3{,}223$, 2,957, 1,665, 1,630, 1,603, 1,441 cm^{-1}; MS (70 eV): $m/z = 351$ (M^+).

2-(3-Nitrobenzoyl)amino-N-(4-methylphenyl)-5-thiazolecarboxamide (**7h**, $C_{18}H_{14}N_4O_4S$)
White powder, yield 0.32 g (40.1 %); m.p.: 274–276 °C; ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 12.63$ (s, 1H, –NH–CO–), 10.11 (s, 1H, –NH–CO–), 7.59 (s, 1H, –C=CH–N=), 7.56 (d, $J = 7.8$ Hz, 1H, Ar–H), 7.34–7.44 (m, 3H, Ar–H), 7.27 (d, $J = 4.2$ Hz, 2H, Ar–H), 7.14 (d, $J = 7.8$ Hz, 2H, Ar–H), 2.27 (s, 3H, –CH_3) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): $\delta = 25.44$ (CH_2), 120.23, 124.26, 126.48, 127.39, 128.95 (2), 129.31, 129.32, 129.80 (2), 131.42, 135.27, 138.79, 141.48, 155.17 (C=O, carboxamide), 155.38 (C=O, carboxamide), 161.11 (S–C=N, thiazole) ppm; IR (KBr): $\bar{\nu} = 3{,}277$, 2,957, 1,661, 1,632, 1,600, 1,500,1,488 cm^{-1}. MS (70 eV): $m/z = 382$ (M^+).

Biologic activity

The human erythroleukemia K562 cell line maintained in suspension culture in Iscove's medium supplemented with 10 % iron-supplemented calf serum, 100 IU/cm^3 penicillin and 0.1 mg/cm^3 streptomycin was added at 37 °C in a 7 % CO_2 incubator. Cells were seeded in triplicate in 96-well plates, at a density of 10^4 cells/well, in the presence of various concentrations of drugs (10^{-5} to 10^{-10} μM) dissolved in dimethyl sulfoxide (DMSO), the concentration of DMSO did not exceed 0.5 % to avoid the effect of DMSO on K562 cells. The cell proliferation assay was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric method after 2 days of incubation; the percentage of growth inhibition IC_{50} was calculated by a comparison with DMSO-treated control cells, which provided the drug.

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