Synthesis, antimicrobial and mitotic toxicity evaluation of new 6-substituted 2-(benzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetic acids

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Abstract: A series of novel 6-substituted 2-(benzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetic acids was synthesized and characterized by ^{1}H, ^{13}C, ^{19}F NMR, ^{1}H-^{1}H-COSY, ^{1}H-^{13}C-HSQC, NOESY, LC-MS, IR and elemental analysis. Mitotic toxicity of the synthesized compounds was determined according to the Allium test procedure. The 2-(6-(pentafluorophenyl)benzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetic acid inhibited mitotic spindle formation, which resulted in significant cytotoxic effect for meristematic cells of *Allium cepa* L. roots. In a preliminary antimicrobial evaluation, only *Streptococcus pyogenes* and *Candida albicans* were slightly susceptible to some of the synthesized compounds.

Introduction

Benzo[4,5]imidazo[1,2-*c*]quinazoline scaffold is of significant focus in the field of medical chemistry due to its broad spectrum of biological properties, ranging from antitumor,[1] anticonvulsant[2] and bronchodilator[3,4] to antimicrobial activities.[5-9] The vast majority of bioactive benzo[4,5]imidazo[1,2-*c*]quinazolines are represented by 6-aryl and 6-hetaryl substituted derivatives. The most common approaches towards their synthesis involve the condensation of anthranilic acid with 1,2-phenylenediamine in polyphosphoric acid (PPA)[8,10] or the cyclodehydrogenation of 2-nitrobenzaldehyde with 1,2-phenylenediamine in the presence of $KHSO_5$, $CeCl_3{\cdot}7H_2O$-CuI-I_2 or air as oxidants, followed by reduction of nitro group.[11-15] The cyclization of the resulting 2-(1*H*-benzo[*d*]imidazol-2-yl)aniline with different aldehydes, acyl chlorides or orthoesters gives 6-substituted 5,6-dihydrobenzo- or benzo[4,5]imidazo[1,2-*c*]quinazolines, respectively.[2,12,16-25] Besides, the fusion of 2-substituted 4*H*-3,1-benzoxazin-4-one with 1,2-phenylenediamine also yields quite good results.[26-28]

It should be noted, that despite the large number of publications on the synthesis of benzo[4,5]imidazo[1,2-*c*]quinazolines their 5,6-disubstituted derivatives are almost unknown. Therefore, this article reveals the synthetic route for aforementioned class of compounds. The designed method was used to introduce carboxymethyl group into the 5-position and various substituents into the 6-position of benzo[4,5]imidazo[1,2-*c*]quinazoline scaffold. The *in vitro* evaluation of the obtained compounds' antimicrobial and mitotic toxicity activities was conducted as well. Moreover, a detailed analysis of the structure of compounds would provide an opportunity to understand the structure-activity relationship and to identify a more advantageous option. Obtained results may be used for purposeful search of chemotherapeutic agents among compounds with cytotoxic activities or finding promising objects for studies aimed at developing of compounds with other types of pharmacological activity among non-cytotoxic products.

Results and Discussion

Chemistry

Initially, commercially available anthranilic acid, as mentioned above, was condensed with 1,2-phenylenediamine in a mixture of concentrated H_3PO_4 and P_4O_{10} at 250°C to afford **1**, which was further treated with carbonyldiimidazole (CDI) to provide intermediate **2** (this compound had been previously obtained by another synthetic method)[29] (Scheme 1). *N*-alkylation of this intermediate by ethyl 2-chloroacetate in the presence of anhydrous K_2CO_3 gave an almost quantitative yield of **3** after the refluxing for a few hours in anhydrous dimethylformamide (DMF). Subsequent hydrolysis of the ester and cyclic urea groups of **3** by treatment with an excess of NaOH in a H_2O-EtOH solution at 80°C afforded **4**. Finally, the target 6-substituted 2-(benzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetic acids **5a-n** (Figure 1) were prepared by refluxing of appropriate aldehyde with **4** in AcOH for 6 hours. During this time the color fluorescence of the solution in UV-A was almost always gradually changing from blue to green. Potassium salts **6a** and **6b** were prepared by treatment of **5k** and **5g**, respectively, with KOH in EtOH.

Particular attention should be given to the clean-up of the reaction products between **4** and (*R*)-2-methylhexanal, which was far less straightforward. It was supposed to be a diastereomeric mixture, but, according to the TLC data, only one component was detected as a product of the reaction, which was unexpected. What is more interesting is that after silica gel column chromatography of the crude product LC-MS analysis gave the single molecular ion peak with desired mass. Nevertheless, ^{1}H and ^{13}C NMR data made it clear that the sample was a mixture composed of two diastereomers in the 1:1 ratio. It is important to emphasize that the chemical shifts were almost identical to each other and often came together. The attempts to separate these diastereomers by column chromatography were unsuccessful.

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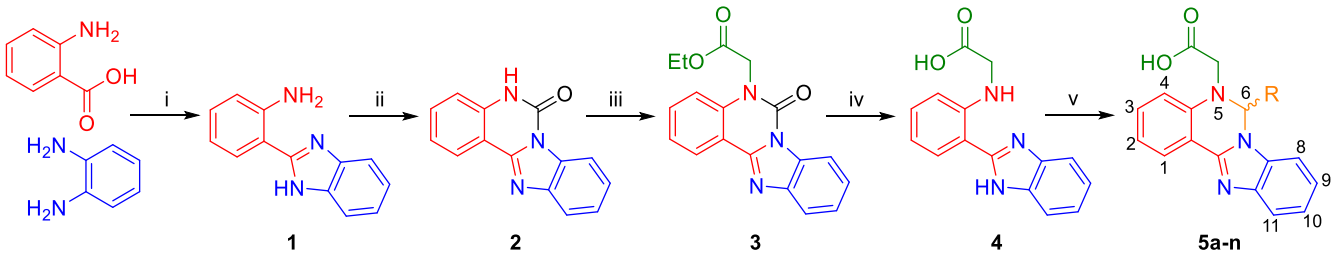

Scheme 1. Synthesis of 6-substituted 2-(benzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetic acids **5a-n**. Reaction conditions: (i) PPA, 250°C, 4 h, 25.3%; (ii) CDI, lioxane, reflux, 2 h, 75.1%; (iii) K_2CO_3, DMF, reflux 1 h, then $ClCH_2COOEt$, reflux, 3 h, 94.9%; (iv) NaOH, H_2O-EtOH, 80°C, 4 h, 92.2%; (v) RCHO, AcOH, reflux, 6 h, 16.8-75.3%.

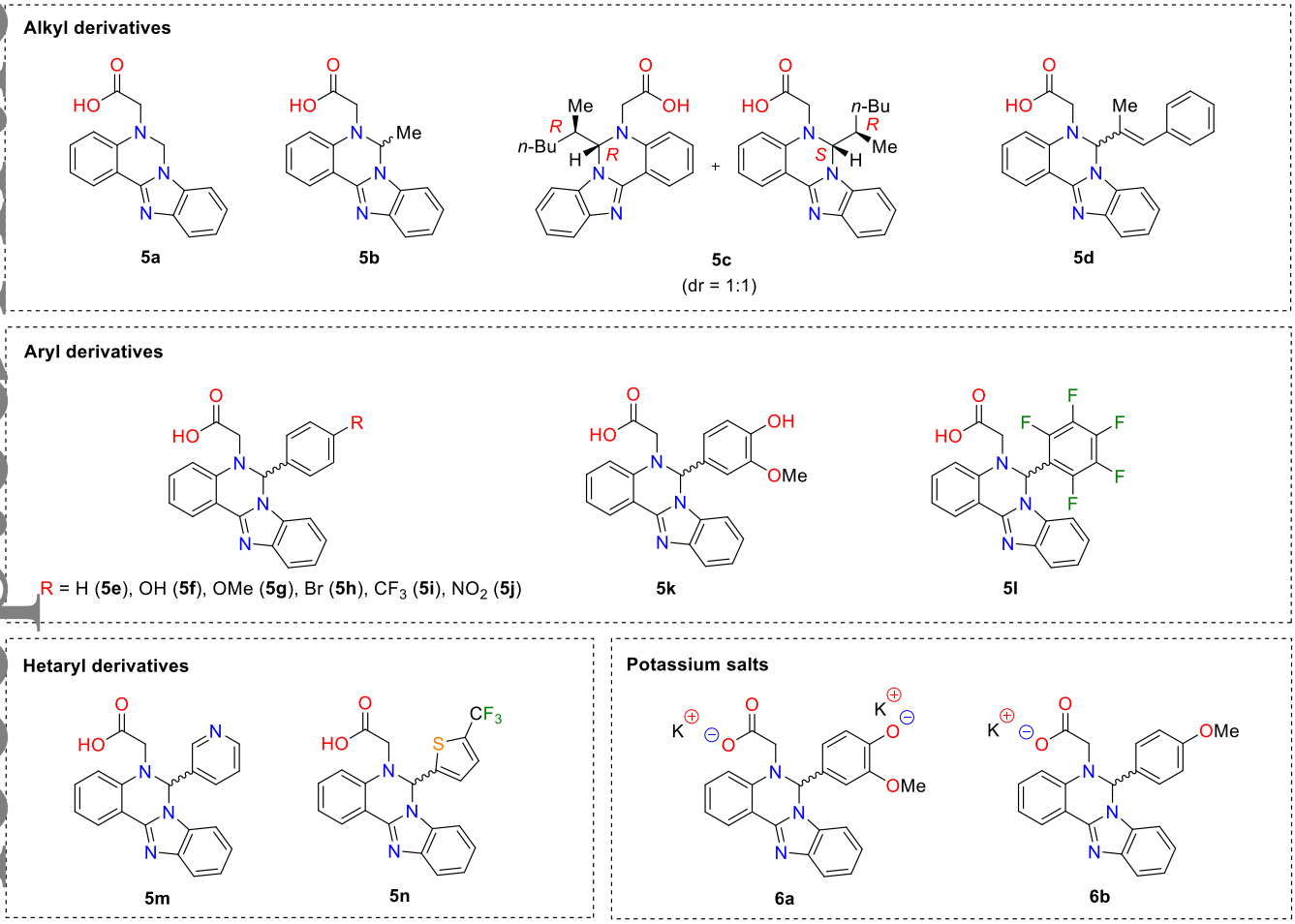

Figure 1. Structures of obtained compounds.

In addition, it was found that ketones did not react with **4**. Thus, acetophenone gave only 2% of the desired product according to the LC-MS data of the residue, while acetone even gave no tracers. In both cases, about 20% of decarboxylation product of **4** was observed. The refluxing of **4** and 4-bromoacetophenone in MeOH with a few drops of conc. HCl as a catalyst for an hour and a half led to a small amount of methyl ester of **4** after column chromatography (20-75% EtOAc-hexane). This leads to the conclusion that the steric hindrance greatly affects reaction in this case.

The chemical structures of all synthesized compounds were verified by spectroscopic methods. Chemical ionization mass spectra of the compounds **2-4** and **5a-n** displayed the correct molecular ions in accordance with the suggested structures. The diastereotopic methylene protons in acetic acid moiety of compounds **5b-n** have a different chemical environment due to the presence of chiral carbon atom at C(6). Hence, signals of above-mentioned protons were registered as a doublet of doublets at the range of 4.15-5.04 ppm with ^{2}J coupling constant of 18.2-19.0 Hz. The ^{13}C NMR-spectra additionally proved the structure of synthesized 6-substituted 2-(benzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetic acids **5a-n**. Thus, the signal of the carbon atom at the 6-position at the range 60.6-78.8 ppm was characteristic and confirmed the formation of partially saturated pyrimidine-containing polycyclic system. In ^{13}C NMR spectrum of compound **5l** the signals of carbon atoms in pentafluorophenyl group were registered as a series of hardly distinct low-intensive multiplets, which melted into the background noise due to the C-F couplings. However, the presence of the C_6F_5 group in **5l** was clearly confirmed by ^{19}F NMR (see Supporting Information File). IR spectra of compounds **5g** and **5k** were characterized by medium-weak absorption bands at 1704-1709 cm^{-1} (C=O str.) and medium-strong ones at 1612-1616 cm^{-1} (COO$^-$ str. as.) and 1395-1392 cm^{-1} (COO$^-$ str. s.). The aforementioned fact makes it possible to assume that compounds **5g** and **5k** might exist in zwitterion form. In IR spectra of potassium salts **6a** and **6b** there are no absorption bands of C=O group, while the COO$^-$ bands became more intense. All of them show strong absorption band at 742 cm^{-1} (C-H$_{aryl}$ bend.), which indicates that they are 1,2-substituted benzenoid compounds.[30] The chemical structure of **5c** was fully characterized based on the ^{1}H-^{1}H-COSY and ^{1}H-^{13}C-HSQC data. The stereochemistry was unambiguously determined by NOESY experiment (see Figure 2 and Supporting Information File).

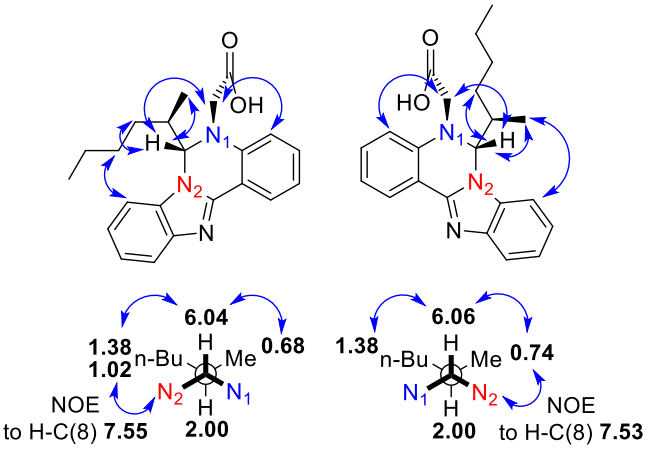

Figure 2. Selected NOE correlations for diastereomeric mixture **5c**.

Biology

The 6-substituted 2-(benzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetic acids **5a-n** and potassium salts **6a** and **6b** were tested *in vitro* for their antibacterial activity against 7 strains of Gram-negative bacteria and 2 strains of Gram-positive bacteria according to the Kirby–Bauer disc diffusion method.[31] From screening result, it was observed that **5c**, **5e**, **5h**, **5l** and **6a** were slightly (12 mm) active against *Streptococcus pyogenes* compared to ciprofloxacin (20 mm) as a reference broad-spectrum antibiotic (Table 1). The antifungal activity was similarly evaluated *in vitro* against *Candida albicans*. This strain showed very small inhibition zones of 9 mm around a **5g** disk and 8 mm around a **5k** disk.

Table 1. Antimicrobial screening results of the tested compounds **5a-n**, **6a** and **6b**.

Compounds	Diameter of the inhibition zone in mm									
	S. aureus	*S. pyogenes*	*P. vulgaris*	*S. typhimurium*	*E. coli*	*P. aeruginosae*	*S. sonnei*	*K. pneumonia*	*K. aerogenes*	*C. albicans*
5a	6	6	6	6	6	6	6	6	6	6
5b	6	6	6	6	6	6	6	6	6	6
5c	6	**12**	6	6	6	6	6	6	6	6
5d	6	6	6	6	6	6	6	6	6	6
5e	6	**12**	6	6	6	6	6	6	6	6
5f	6	6	6	6	6	6	6	6	6	6
5g	6	6	6	6	6	6	6	6	6	**9**
5h	6	**12**	6	6	6	6	6	6	6	6

5i	6	6	6	6	6	6	6	6	6	6
5j	6	6	6	6	6	6	6	6	6	6
5k	6	6	6	6	6	6	6	6	6	**8**
5l	6	**12**	6	6	6	6	6	6	6	6
5m	6	6	6	6	6	6	6	6	6	6
5n	6	6	6	6	6	6	6	6	6	6
6a	6	**12**	6	6	6	6	6	6	6	6
6b	6	6	6	6	6	6	6	6	6	6
Ciprofloxacin	18	20	32	37	30	35	28	30	27	-
Ketoconazole	-	-	-	-	-	-	-	-	-	17

A few interesting features of the structure-activity relationship were discovered. Thus, according to the reported study 6-(pyridine-3-yl)benzo[4,5]imidazo[1,2-*c*]quinazoline was active against a number of bacteria and fungi,[8] while 2-(6-(pyridin-3-yl)benzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetic acid (**5m**) was not (Figure 3A). Apparently, the partial saturation benzo[4,5]imidazo[1,2-c]quinazoline fragment as well as introduction of acetic acid moiety into the 5-position led to the loss of antimicrobial activity. Additional introduction of aryl fragment with methoxy group at the para position (**5g**) caused the occurrence of growth inhibition activity towards to *C. albicans* strain. The replacement of methoxy group by hydroxyl group (**5f**) resulted in the loss of antifungal activity. At the same time, compound **5k** that contained both as methoxy and hydroxyl groups in aryl fragment turned out to have caused a slightly higher antifungal activity than **5g**. The introduction of long aliphatic fragment (**5c**) and benzene ring (**5e**) into the 6-position of heterocyclic system resulted in the appearance of activity against *S. pyogenes*. The introduction of oxygen-containing groups into the aryl fragment at the 6-position (**5f**, **5g**, **5k**) and substitution of aryl fragment by heterocyclic moiety (**5m**, **5n**) decreased the activity against above-mentioned strain. The replacement of the carboxy and hydroxyl groups in **5k** by their potassium salts (**6a**) decreased antifungal activity and significantly increased activity against *S. pyogenes*. It should be noted that compound **5k** as well as jatrorrhizine comprised (4-hydroxy-3-methoxybenzyl)(methyl)amino fragment and both showed a weak antifungal activity,[32] which implies that this fragment is a key structural component for further studies to develop new antifungal agents (Figure 3B).

Subsequently the mitotic toxicity of synthesized 6-substituted 2-(benzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetic acids **5a-n** were examined according to the Allium test procedure.[33,34] The above-mentioned method was chosen due to its conventional application for screening of mutagenic and cytotoxic effects of chemical factors, availability and reproducibility. Moreover, this test was described as an effective drug safety evaluation method.[35] Methotrexate (**m**) was used as a reference agent,[36] while tap water (**w**) and KOH (**k**) – as intact ones. Figure 4 shows images of the bulbs and root length taken at the beginning of the experiment and at the end of days 1, 2 and 3.

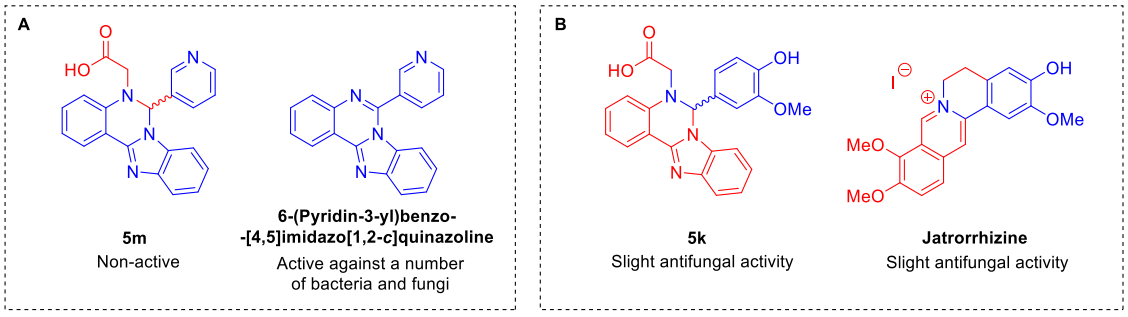

Figure 3. The structure-activity relationship between the new synthesized compounds and previously reported ones.

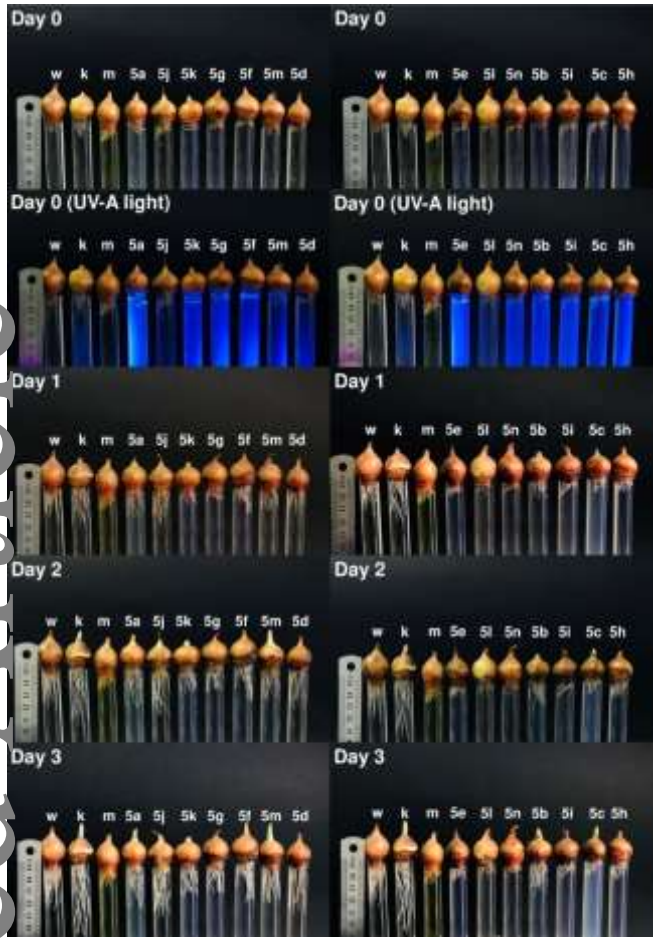

Figure 4. Growth retardation effects of compounds **5a-n**. **w** tap water, **k** 0.001 N KOH in tap water, **m** 1 mmol/l methotrexate in tap water with 0.002 N KOH, **5a-n** 1 mmol/l **5a-n** in tap water with 0.001 N (0.002 N for **5f** and **5k**) KOH.

First of all, it is noteworthy that **m** led to a complete inhibition of root growth of *Allium cepa* L., thus, the total increase of root length in 3 days was 0 mm, while it was 25 mm in **w** and 47 mm in **k** (Table 2). The microscopic study showed that under the action of methotrexate meristematic root tip cells undergo some degenerative changes, such as karyopyknosis and cell fragmentation (Figure 5F). Compounds **5c**, **5e**, **5h**, **5i**, **5l** and **5n** exhibited a significant growth inhibition effect. The analysis of the structure-roots growth inhibition activity relationship revealed that the introduction of halogen or halogen-containing group into aryl (hetaryl) fragment at the 6-position had promoted the growth inhibitory activity of obtained compounds. On the one hand, mitotic index of **5c**, **5i** and **5n** was the same as for methotrexate, namely equal to 0.0% (Table 3). On the other hand, the severe degenerative changes were induced by **5i**, **5n** (karyolysis) and especially **5c** (karyolysis and extensive cell fragmentation), which indicated a highly toxic effect (Figure 5G).

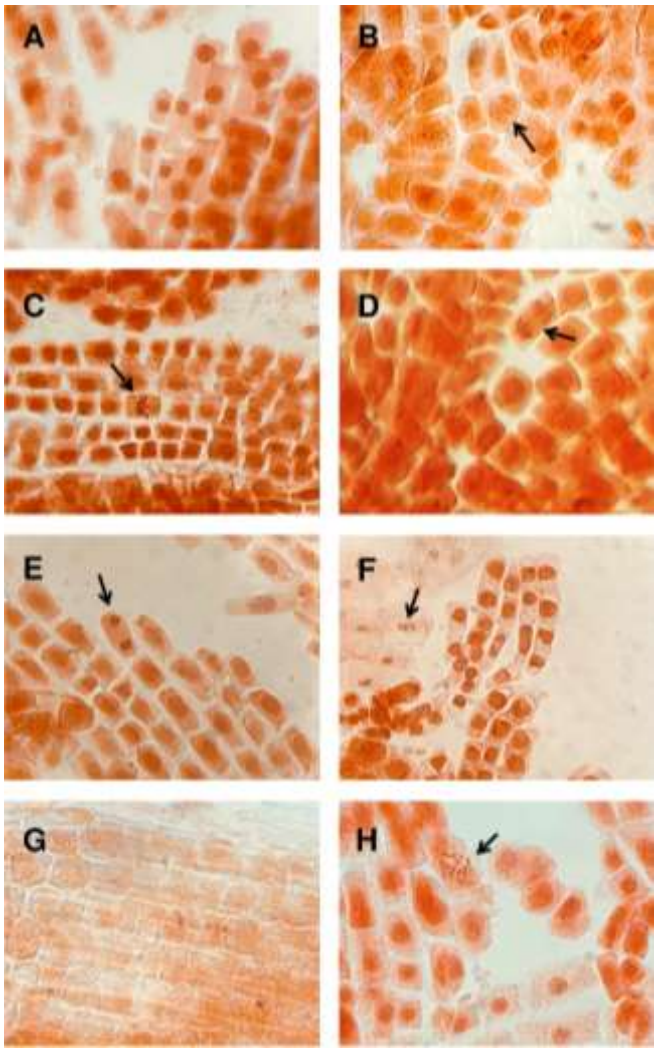

Figure 5. Cellular alterations observed by analysing meristematic cells of *Allium cepa* L. roots. Treatment in parentheses. **A** normal interphase (tap water). **B** normal prophase (tap water), **C** normal methaphase (tap water), **D** normal anaphase (tap water), **E** normal telophase (tap water), **F** karyopyknosis (methotrexate), **G** karyolysis (**5c**), **H** c-mitosis (**5l**) (600×).

Table 2. Roots lengths of *Allium cepa* L. after 3 days of cultivation in 1 mmol/l **5a-n** aqueous solution with 0.001 N (0.002 N for **5f** and **5k**) KOH.

Compounds 1 mmol/l	Root length in mm				Total increase of root length in mm
	Day 0	Day 1	Day 2	Day 3	
5a	10	22	31	36	26

5b	5	13	18	24	19
5c	9	10	10	10	**1**
5d	6	18	30	37	31
5e	5	7	10	12	**7**
5f	10	22	25	31	21
5g	5	11	18	23	18
5h	5	8	11	12	**7**
5i	10	11	12	13	**3**
5j	8	29	37	40	32
5k	5	12	19	26	21
5l	6	6	8	10	**4**
5m	8	16	21	30	22
5n	6	7	11	11	**5**
w[a]	8	23	31	33	25
k[b]	10	32	46	57	47
m[c]	9	9	9	9	0

[a] Tap water. [b] 0.001 N KOH in tap water. [c] 1 mmol/l methotrexate in tap water with 0.002 N KOH.

Since c-mitosis have been observed in root tips that had been exposed to phenyl (**5e**) and pentafluorophenyl (**5l**) derivatives, these compounds are of particular interest as potential chemotherapeutic agents (Figure 5H). Moreover, any other abnormalities of root apex cells were not detected. C-mitosis is characterized by well separated metaphase chromosomes dispersed in the cell, not oriented along the equatorial plate. The occurrence of c-mitosis indicates that **5e** and **5l** have caused the inhibition of spindle formation, similar to the effect of colchicine.[37] Compared to tap water, **5e** and **5l** reduced the mitotic index by 57.14% and 81.63%, respectively. The percentage of cells in c-mitosis for **5e** and **5l** was closely correlated to their mitotic index values and was 14.3% and 33.3%, respectively. Thus, it can be concluded that the replacement of all hydrogen atoms of phenyl group at the 6-position by fluorine atoms considerably increased the inhibition of spindle formation.

Table 3. Mitotic toxicity of compounds **5a-n**.

Compounds 1 mmol/l	Total cells counted	Cells in mitotic stage	Mitotic index (%)	C-mitosis (%)
5a	632	22	3.5	0.0
5b	691	26	3.8	0.0
5c	617	**0**	**0.0**	0.0
5d	637	25	3.9	0.0
5e	660	**14**	**2.1**	**14.3**
5f	624	23	3.7	0.0
5g	673	24	3.6	0.0

5h	679	**13**	**1.9**	0.0
5i	602	**0**	**0.0**	0.0
5j	618	29	4.7	0.0
5k	651	25	3.8	0.0
5l	645	**6**	**0.9**	**33.3**
5m	645	23	3.6	0.0
5n	611	**0**	**0.0**	0.0
w[a]	658	32	4.9	0.0
k[b]	610	39	6.4	0.0
m[c]	624	0	0.0	0.0

[a] Tap water. [b] 0.001 N KOH in tap water. [c] 1 mmol/l methotrexate in tap water with 0.002 N KOH.

Any chromosome aberrations, abnormalities in the mitotic cycle or degenerative changes to the other synthesized compounds were not detected. As we consider, compounds that have not revealed anti-mitotic activity are interesting objects for screening of types of biological activities, which are not associated with inhibition of cell growth.

Conclusions

The convenient method for synthesis of 5,6-disubstituted benzo[4,5]imidazo[1,2-*c*]quinazolines based on sequential alkylation of benzo[4,5]imidazo[1,2-*c*]quinazolin-6(5*H*)-one, hydrolytic cleavage of pyrimidone fragment and two component heterocyclocondensation with carbonyl-containing reagents has been elaborated. The structures of the obtained 6-substituted 2-(benzo[4,5]imidazo[1,2-c]quinazolin-5(6*H*)-yl)acetic acids have been confirmed by a modern physicochemical method. The preliminary screening for antimicrobial activity and mitotic toxicity of the obtained compounds has been conducted. It has been established, that 6-substituted 2-(benzo[4,5]imidazo[1,2-c]quinazolin-5(6*H*)-yl)acetic acids are not active against most of the studied strains, but some of the synthesized compounds have been slightly active against *S. pyogenes* (**5c**, **5e**, **5h**, **5l**, **6a**) and *C. albicans* (**5g**, **5k**). According to the Allium test result the substance **5k** or its dipotassium salt **6a** showed no significant mitotic toxicity effect. The pentafluorophenyl derivative **5l** displayed potent *in vitro* cytotoxic effect (inhibition of mitotic spindle formation) for root meristematic cells of *Allium cepa* L. and it showed an activity close to that of the positive control methotrexate. The analysis of structure-biological activity correlations made it possible to detect the fragments that are essential for occurrence of the proper type of biological activity. The absence of mitotic toxicity of some of the obtained compounds showed high prospects of their further screening for other types of biological activity.

Experimental Section

Chemistry

The substance **1** was synthesized according to the reported procedure.[10] All commercially available chemicals were used without further purification. Melting points were uncorrected. IR spectra were recorded on a Shimadzu IR Prestige-21 Fourier spectrometer fitted with an attenuated total reflectance sampling accessory. The elemental analyses (C, H, and N) were performed using the Elementar vario EL cube analyzer. NMR spectra were measured on a Bruker DRX 500 (400 or 500 MHz, ^{1}H; 100, 125 or 150 MHz, ^{13}C; 470 MHz, ^{19}F). Tetramethylsilane (^{1}H, ^{13}C) or $CFCl_3$ (^{19}F) were used as an internal reference for the spectra. The chemical shift values (δ) and coupling constants (*J*) are expressed in parts per million (ppm) and hertz (Hz), respectively. Chemical shifts were referenced to residual non deuterated solvent (dimethyl sulfoxide (DMSO) ^{1}H: δ = 2.50 ppm, ^{13}C: δ = 40.0 ppm and trifluoroacetic acid (TFA) ^{1}H: δ = 11.18 ppm, ^{13}C: δ = 153.0, 105.7 ppm). Column chromatography was carried out using Merck silica gel 60 (0.040-0.063 mm) as the stationary phase and analytical TLC was performed using Merck silica gel 60 F_{254} aluminium sheets. Mass spectra were recorded with an LC-MS instrument using chemical ionization (CI). LC-MS data were acquired with an Agilent 1200 HPLC system equipped with a DAD/ELSD/LCMS-6120 diode matrix and mass-selective detector, column: Poroshell 120 SBC18, 4.6×30 mm; eluent A: MeCN-H_2O 99:1 with 0.1% of HCO_2H; eluent B: H_2O with 0.1% of HCO_2H.

Benzo[4,5]imidazo[1,2-*c*]quinazolin-6(5*H*)-one (2)

13.0 g (62.1 mmol) of **1** and 11.5 g (70.9 mmol) of CDI in 75 ml of dioxane were refluxed for 2 h. The reaction mixture was poured into 200 ml of H_2O and neutralized with conc. HCl, making solution slightly acid. The precipitate was filtered, washed with H_2O and dried at 60°C to provide a white-gray solid. Yield: 10.93 g (75.1%); m.p. >300°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.95 (s, 1H), 8.36 (d, ^{3}J = 7.9 Hz, 1H), 8.31 (d, ^{3}J = 8.0 Hz, 1H), 7.86 (d, ^{3}J = 7.9 Hz, 1H), 7.65 (t, ^{3}J = 7.7 Hz, 1H), 7.55 – 7.29 (m, 4H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 148.1, 146.8, 144.0,

137.6, 132.7, 131.1, 125.5, 124.9, 124.1, 123.8, 119.6, 116.4, 115.2, 112.3; MS (CI): *m/z* 236.1 $[M + H]^+$; Anal. Calcd. for $C_{14}H_9N_3O$: C, 71.48; H, 3.86; N, 17.86; Found: C, 71.41; H, 3.89; N, 17.91.

Ethyl 2-(6-oxobenzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetate (3)

10.93 g (46.5 mmol) of **2** and 3.17 g (22.9 mmol) of anhydrous K_2CO_3 in 75 ml of dry DMF were refluxed for 1 h. 5.47 g (44.6 mmol) of ethyl 2-chloroacetate in 10 ml of dry DMF was added dropwise to the stirring mixture. The reaction mixture was refluxed for 3 h and poured into 500 ml of H_2O. The precipitate was filtered, washed with H_2O and dried at 60°C to afford a white solid. Yield: 14.17 g (94.9%); m.p. 207°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.49 (d, ^3J = 7.7 Hz, 1H), 8.38 (d, ^3J = 8.0 Hz, 1H), 7.81 (d, ^3J = 7.7 Hz, 1H), 7.69 (t, ^3J = 8.3 Hz, 1H), 7.52 – 7.37 (m, 4H), 5.15 (s, 2H), 4.26 (q, ^3J = 7.1 Hz, 2H), 1.32 (t, ^3J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.5, 147.0, 147.0, 143.9, 137.7, 133.2, 131.1, 125.9, 125.5, 124.6, 124.5, 119.8, 115.8, 115.2, 113.0, 61.9, 45.1, 14.5; MS (CI): *m/z* 322.2 $[M + H]^+$; Anal. Calcd. for $C_{18}H_{15}N_3O_3$: C, 67.28; H, 4.71; N, 13.08; Found: C, 67.24; H, 4.68; N, 13.12.

(2-(1*H*-Benzo[*d*]imidazol-2-yl)phenyl)glycine (4)

14.17 g (44.1 mmol) of **3** and 26.5 g (0.66 mol) of NaOH in 310 ml of H_2O-EtOH 85:15 were heated at 80 °C for 4 h with stirring. The resulting solution was filtered hot and 53 ml (determined by titration of starting NaOH solution, using phenolphthalein as an internal indicator) of conc. HCl was added. The precipitate was filtered, washed with H_2O and dried at 60°C to give a white-yellow solid. Yield: 10.87 g (92.2%); m.p. 145°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.68 (s, 1H), 9.42 (s, 1H), 7.90 (d, ^3J = 7.0 Hz, 1H), 7.62 – 7.51 (m, 2H), 7.24 (t, ^3J = 7.1 Hz, 1H), 7.21 – 7.14 (m, 2H), 6.71 (t, ^3J = 7.5 Hz, 1H), 6.63 (d, ^3J = 8.4 Hz, 1H), 4.08 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.6, 152.6, 147.5, 131.4, 128.0, 122.5, 115.7, 111.6, 111.4, 45.0; MS (CI): *m/z* 268.0 $[M + H]^+$; Anal. Calcd. for $C_{15}H_{13}N_3O_2$: C, 67.40; H, 4.90; N, 15.72; Found: C, 67.46; H, 4.86; N, 15.76.

General procedure for the synthesis of compounds 5a-n

2.6 mmol of **4** and 3.1 mmol of appropriate carbonyl compound in 10 ml of AcOH were refluxed for 6 h (**5a** and **5f** fell out of AcOH during the reaction and after cooling the precipitate was collected by suction filtration). After evaporation *in vacuo*, the residue was treated with MeOH. The precipitate was filtered, washed with MeOH and dried at 60°C to afford the desired product at >90% purity (**5c** was very soluble in MeOH, so the residue after evaporation of solvent was purified by column chromatography (SiO_2, DCM-EtOH 6:4) to give inseparable diastereomeric mixture in a 1:1 ratio).

2-(Benzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetic acid (5a)

Yield: 0.39 g (53.4%) as a light yellow solid; m.p. 265°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.03 (d, ^3J = 7.6 Hz, 1H), 7.68 (d, ^3J = 7.4 Hz, 1H), 7.53 (d, ^3J = 7.5 Hz, 1H), 7.38 (t, ^3J = 7.9 Hz, 1H), 7.30 – 7.17 (m, 2H), 6.97 (t, ^3J = 7.4 Hz, 1H), 6.81 (d, ^3J = 8.3 Hz, 1H), 5.68 (s, 2H), 4.33 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.6, 147.2, 144.7, 144.0, 133.1, 132.1, 125.5, 122.8, 122.7, 119.7, 119.1, 114.4, 113.4, 110.3, 60.6, 50.6; MS (CI): *m/z* 280.2 $[M + H]^+$; Anal. Calcd. for $C_{16}H_{13}N_3O_2$: C, 68.81; H, 4.69; N, 15.05; Found: C, 68.87; H, 4.64; N, 15.09.

2-(6-Methylbenzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetic acid (5b)

Yield: 0.38 g (50.0%) as a brown solid; m.p. 231°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.89 (s, 1H), 8.02 (d, ^3J = 7.6 Hz, 1H), 7.67 (d, ^3J = 7.0 Hz, 1H), 7.52 (d, ^3J = 7.4 Hz, 1H), 7.37 (t, ^3J = 7.9 Hz, 1H), 7.30 – 7.19 (m, 2H), 6.93 (t, ^3J = 7.5 Hz, 1H), 6.77 (d, ^3J = 8.3 Hz, 1H), 6.31 (q, ^3J = 5.9 Hz, 1H), 4.35 (d, ^2J = 18.2 Hz, 1H), 4.26 (d, ^2J = 18.3 Hz, 1H), 1.35 (d, ^3J = 5.9 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.7, 146.3, 144.2, 142.4, 132.4, 132.2, 125.3, 122.7, 122.7, 119.2, 119.2, 114.5, 114.0, 110.1, 68.3, 51.2, 19.6; MS (CI): *m/z* 294.0 $[M + H]^+$; Anal. Calcd. for $C_{17}H_{15}N_3O_2$: C, 69.61; H, 5.15; N, 14.33; Found: C, 69.57; H, 5.10; N, 14.37.

2-((*R*)-6-((*R*)-Hexan-2-yl)benzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetic acid with 2-((*S*)-6-((*R*)-hexan-2-yl)benzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetic acid (1:1) (5c)

Yield: 0.16 g (16.8%) as a light brown solid; R_f = 0.80 (DCM-EtOH 6:4); m.p. 70°C (softens), 81-87°C (melts); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.98 (d, ^3J = 7.7 Hz, 1H, H-C(1)), 7.65 (d, ^3J = 7.5 Hz, 1H, H-C(11)), 7.55 (d, ^3J = 7.5 Hz, 1H, H-C(8)), 7.53 (d, ^3J = 8.3 Hz, 1H, H-C(8)), 7.33 (t, ^3J = 7.9 Hz, 1H, H-C(3)), 7.25 – 7.17 (m, 2H, H-C(9), H-C(10)), 6.97 (t, ^3J = 8.5 Hz, 1H, H-C(4)), 6.91 (t, ^3J = 6.1 Hz, 1H, H-C(2)), 6.06 (d, ^3J = 5.1 Hz, 1H, H-C(6)), 6.04 (d, ^3J = 5.6 Hz, 1H, H-C(6)), 4.58 (d, ^2J = 14.6 Hz, 1H, CH_2COOH), 4.53 (d, ^2J = 14.6 Hz, 1H, CH_2COOH), 4.18 (d, ^2J = 18.2 Hz, 1H, CH_2COOH), 4.15 (d, ^2J = 18.2 Hz, 1H, CH_2COOH), 2.06 – 1.94 (m, 1H, CHCH$_3$), 1.44 – 1.30 (m, 1H, CHCH_2), 1.25 – 1.15 (m, 1H, CHCH_2), 1.04 – 1.00 (m, 4H, CH_2CH_2CH$_3$), 0.77 – 0.65 (m, 6H, CHCH_3, CH$_2$CH_3); ^{13}C NMR (150 MHz, DMSO-d_6): δ 171.9 (COOH), 147.3 (N(7)-C-N(12)), 147.2 (N(7)-C-N(12)), 144.2 (C-N(7)), 144.1 (C-N(7)), 144.0 (C-N(12)), 143.9 (C-N(12))), 133.8 (C-N(5)), 133.6 (C-N(5)), 131.8 (C(3)), 131.8 (C(3)), 125.0 (C(1)), 122.3 (C(9)), 122.2 (C(9)), 122.1 (C(10)), 122.0 (C(10)), 119.5 (C(2)), 119.4 (C(2)), 119.0 (C(11)), 118.9 (C(11)), 116.4 (C-C(1)), 116.3 (C-C(1)), 115.7 (C(4)), 111.0 (C(8)), 110.8 (C(8)), 75.6 (C(6)), 75.4 (C(6)), 54.8 (*C*H$_2$COOH), 54.6 (*C*H$_2$COOH), 41.0 (*C*HCH$_3$), 40.9 (*C*HCH$_3$), 31.5 (*C*H$_2$CH$_3$), 31.4 (CH*C*H$_2$), 28.9 (*C*H$_2$CH$_3$), 28.8 (CH*C*H$_2$), 22.6 (*C*H$_2$CH$_2$CH$_3$), 22.5 (*C*H$_2$CH$_2$CH$_3$), 15.3 (CH*C*H$_3$), 15.0 (CH*C*H$_3$), 14.1 (CH$_2$*C*H$_3$), 14.0 (CH$_2$*C*H$_3$); ^{1}H-^{1}H-COSY cross-peaks: 6.91/7.98 (H-C(2)/H-C(1)), 7.21/7.65 (H-C(10)/H-C(11)), 7.21/7.54 (H-C(9)/H-C(8)), 6.97/7.33 (H-C(4)/H-C(3)), 4.16/4.55 (CH*H*`COOH/C*H*H`COOH), 2.00/6.05 (C*H*CH$_3$/H-C(6)), 1.19/1.38 (CH*CH*H`/CHCH*H*`),1.02/1.38 (C*H*$_2$CH$_2$CH$_3$/CHCH*H*`), 1.02/1.19 (C*H*$_2$CH$_2$CH$_3$/CHC*H*H`), 0.74/2.00 (CHC*H*$_3$/C*H*CH$_3$), 0.68/2.00 (CHC*H*$_3$/C*H*CH$_3$), 0.70/1.02 (CH$_2$C*H*$_3$/C*H*$_2$CH$_3$); ^{1}H-^{13}C-HSQC cross-peaks:7.33/131.9 (H-C(3)/C(3)), 7.98/124.9 (H-C(1)/C(1)), 7.21/122.3 (H-C(9), H-C(10)/C(9), C(10)), 7.65/119.0 (H-C(11)/C(11)), 6.91/119.3 (H-C(2)/C(2)), 6.97/115.6 (H-C(4)/C(4)), 7.54/110.8 (H-C(8)/C(8)), 6.05/75.5 (H-C(6)/C(6)), 4.55/54.8 (C*H*H`COOH/ C*H*H`COOH), 4.17/54.7 (CH*H*`COOH/C*H*H`COOH), 2.00/41.1 (C*H*CH$_3$/C*H*CH$_3$), 1.02/31.6 (C*H*$_2$CH$_3$/C*H*$_2$CH$_3$), 1.38/31.5 (CHC*H*$_2$/CHC*H*$_2$), 1.02/29.0 (C*H*$_2$CH$_3$/C*H*$_2$CH$_3$), 1.19/28.9 (CHC*H*$_2$/CHC*H*$_2$), 1.06/22.7 (C*H*$_2$CH$_2$CH$_3$/CH$_2$CH$_2$CH$_3$), 0.68/15.2 (CHC*H*$_3$/CHC*H*$_3$), 0.72/14.9 (CHC*H*$_3$/CHC*H*$_3$), 0.71/12.7 (CH$_2$C*H*$_3$/CH$_2$C*H*$_3$); NOESY cross-peaks: 6.91/7.98 (H-C(2)/ H-C(1)), 7.21/7.65 (H-C(10)/ H-C(11)), 7.21/7.53-7.55 (H-C(9)/H-C(8)), 6.04-6.06/7.53-7.55(H-C(6)/H-C(8)), 6.97/7.33 (H-C(4)/ H-C(3)), 6.91/7.33 (H-C(2)/H-C(3)), 4.53/6.97 (C*H*H`COOH/H-C(4)), 4.15-4.18/ 6.04-6.06 (CH*H*`COOH/ H-C(6)), 4.15-4.18/4.53-4.58 (CH*H*`COOH/ C*H*H`COOH), 2.00/6.04-6.06 (C*H*CH$_3$/H-C(6)), 1.38/6.04-6.06 (CHC*H*$_2$/H-C(6)), 1.38/2.00 (CHC*H*$_2$/C*H*CH$_3$), 1.02/7.55 (C*H*$_2$CH$_2$CH$_3$/ H-C(8)), 1.02/6.04-6.06 (C*H*$_2$CH$_2$CH$_3$/ H-C(6)), 1.02/2.00 (C*H*$_2$CH$_2$CH$_3$/C*H*CH$_3$), 1.02/1.38 (C*H*$_2$CH$_2$CH$_3$/C*H*CH$_2$), 0.74/7.53 (CHC*H*$_3$/H-C(8)), 0.68/7.55 (CHC*H*$_3$/H-C(8)), 0.74/6.06 (CH$_2$C*H*$_3$/H-C(6)), 0.68/6.04 (CH$_2$C*H*$_3$/H-C(6)), 1.38/2.00 (CHC*H*$_2$/C*H*CH$_3$), 1.02/2.00 (C*H*$_2$CH$_2$CH$_3$/C*H*CH$_3$), 0.74/2.00 (CHC*H*$_3$/C*H*CH$_3$), 0.68/2.00 (CHC*H*$_3$/C*H*CH$_3$), 0.70/1.19 (CH$_2$C*H*$_3$/CHC*H*$_2$), 0.70/1.08-1.02 (CH$_2$C*H*$_3$/C*H*$_2$CH$_2$CH$_3$); MS (CI): *m/z* 364.2 $[M + H]^+$; Anal. Calcd. for

$C_{22}H_{25}N_3O_2$: C, 72.70; H, 6.93; N, 11.56; Found: C, 72.65; H, 6.97; N, 11.62.

(*E*)-2-(6-(1-Phenylprop-1-en-2-yl)benzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetic acid (5d)

Yield: 0.50 g (48.5%) as a green solid; m.p. 255°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.81 (s, 1H), 8.04 (d, ^3J = 7.8 Hz, 1H), 7.69 (d, ^3J = 6.2 Hz, 1H), 7.52 – 7.44 (m, 1H), 7.36 – 7.31 (m, 3H), 7.30 – 7.19 (m, 5H), 7.04 (s, 1H), 6.88 (t, ^3J = 7.3 Hz, 1H), 6.78 (d, ^3J = 8.3 Hz, 1H), 6.64 (s, 1H), 4.39 (d, ^2J = 18.5 Hz, 1H), 4.28 (d, ^2J = 18.2 Hz, 1H), 1.38 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.3, 146.5, 144.3, 143.0, 136.4, 134.5, 132.8, 132.3, 129.5, 129.4, 128.8, 127.7, 125.1, 123.0, 122.7, 119.2, 118.4, 112.8, 111.9, 111.0, 78.8, 49.9, 12.0; MS (CI): *m/z* 396.2 [M + H]$^+$; Anal. Calcd. for $C_{25}H_{21}N_3O_2$: C, 75.93; H, 5.35; N, 10.63; Found: C, 75.98; H, 5.41; N, 10.69.

2-(6-Phenylbenzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetic acid (5e)

Yield: 0.57 g (61.2%) as a brown solid; m.p. 259°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.81 (s, 1H), 8.10 (d, ^3J = 7.7 Hz, 1H), 7.68 (d, ^3J = 8.0 Hz, 1H), 7.36 (t, ^3J = 7.3 Hz, 1H), 7.32 (d, ^3J = 7.7 Hz, 1H), 7.29 – 7.23 (m, 5H), 7.22 – 7.18 (m, 2H), 7.15 (t, ^3J = 7.6 Hz, 1H), 6.96 (t, ^3J = 7.5 Hz, 1H), 6.82 (d, ^3J = 8.4 Hz, 1H), 4.30 (d, ^2J = 18.2 Hz, 1H), 4.22 (d, ^2J = 18.2 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 171.0, 146.5, 144.2, 142.6, 138.9, 132.9, 132.3, 129.6, 129.3, 126.3, 125.4, 122.9, 122.7, 119.5, 119.2, 114.2, 114.0, 110.6, 73.0, 50.7; MS (CI): *m/z* 356.0 [M + H]$^+$; Anal. Calcd. for $C_{22}H_{17}N_3O_2$: C, 74.35; H, 4.82; N, 11.82; Found: C, 74.29; H, 4.78; N, 11.86.

2-(6-(4-Hydroxyphenyl)benzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetic acid (5f)

Yield: 0.66 g (68.0%) as a light yellow solid; m.p. 281°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.79 (s, 1H), 9.64 (s, 1H), 8.12 (d, ^3J = 7.7 Hz, 1H), 7.68 (d, ^3J = 8.0 Hz, 1H), 7.37 (t, ^3J = 7.8 Hz, 1H), 7.25 – 7.18 (m, 2H), 7.17 – 7.11 (m, 3H), 7.06 (s, 1H), 6.96 (t, ^3J = 7.5 Hz, 1H), 6.81 (d, ^3J = 8.4 Hz, 1H), 6.68 (d, ^3J = 8.2 Hz, 2H), 4.25 (d, ^2J = 18.2 Hz, 1H), 4.16 (d, ^2J = 18.2 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.0, 158.7, 146.5, 144.0, 142.9, 132.9, 132.4, 129.1, 128.1, 125.5, 122.9, 122.7, 119.3, 119.1, 116.0, 113.9, 113.6, 110.9, 73.1, 50.1; MS (CI): *m/z* 372.2 [M + H]$^+$; Anal. Calcd. for $C_{22}H_{17}N_3O_3$: C, 71.15; H, 4.61; N, 11.31; Found: C, 71.19; H, 4.53; N, 11.39.

2-(6-(4-Methoxyphenyl)benzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetic acid (5g)

Yield: 0.47 g (47.0%) as a light yellow solid; m.p. 258°C; IR: ṽ = 3406 (O-H, str., br.), 3005 (C-H$_{aryl}$, str., m.), 2929 – 2835 (C-H$_{alkyl}$, str., m.), 1900 (aromatic overtone), 1704 (C=O, str., m.), 1612 (COO$^-$, str., as., m.), 1571 – 1499 (C=C$_{aryl}$, str., v.), 1460 (C-H$_{alkyl}$, bend., m.), 1395 (COO$^-$, str., s., m.), 1242 (C-O, str., s.), 1174 – 1029 (C-N, str., v.), 828 (1,4-subst. C-H$_{aryl}$, bend., m.), 742 cm^{-1} (1,2-subst. C-H$_{aryl}$, bend., s.); ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.81 (s, 1H), 8.10 (d, ^3J = 7.6 Hz, 1H), 7.67 (d, ^3J = 7.8 Hz, 1H), 7.36 (t, ^3J = 7.8 Hz, 1H), 7.27 (d, ^3J = 7.8 Hz, 1H), 7.22 – 7.18 (m, 3H), 7.15 (t, ^3J = 7.5 Hz, 1H), 7.12 (s, 1H), 6.96 (t, ^3J = 7.4 Hz, 1H), 6.83 (d, 2H), 6.80 (d, ^3J = 8.6 Hz, 1H), 4.25 (d, ^2J = 18.2 Hz, 1H), 4.19 (d, ^2J = 18.3 Hz, 1H), 3.66 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.0, 160.2, 146.5, 144.3, 142.7, 132.9, 132.3, 130.9, 127.9, 125.4, 122.9, 122.7, 119.3, 119.2, 114.6, 114.0, 113.8, 110.8, 72.8, 55.5, 50.3; MS (CI): *m/z* 386.2 [M + H]$^+$; Anal. Calcd. for $C_{23}H_{19}N_3O_3$: C, 71.68; H, 4.97; N, 10.90; Found: C, 71.61; H, 4.93; N, 10.95.

2-(6-(4-Bromophenyl)benzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetic acid (5h)

Yield: 0.57 g (50.4%) as a brown solid; m.p. 274°C; ^{1}H NMR (400 MHz, TFA-*d*): δ 8.52 (d, ^3J = 8.0 Hz, 1H), 8.27 (d, ^3J = 8.3 Hz, 1H), 8.20 (t, ^3J = 7.9 Hz, 1H), 8.12 (t, ^3J = 7.8 Hz, 1H), 8.08 – 7.97 (m, 3H), 7.86 (d, ^3J = 8.3 Hz, 1H), 7.79 (d, ^3J = 7.9 Hz, 2H), 7.73 (t, ^3J = 7.8 Hz, 1H), 7.66 (s, 1H), 7.55 (d, ^3J = 8.3 Hz, 1H), 5.04 (d, ^2J = 19.0 Hz, 1H), 4.92 (d, ^2J = 19.0 Hz, 1H); ^{13}C NMR (100 MHz, TFA): δ 166.4, 135.0, 134.5, 128.8, 124.2, 122.0, 120.6, 119.2, 119.0, 118.4, 117.1, 117.0, 113.6, 106.7, 104.8, 103.2, 98.0, 66.0, 41.6; MS (CI): *m/z* 434.0 [M + H]$^+$; Anal. Calcd. for $C_{22}H_{16}BrN_3O_2$: C, 60.84; H, 3.71; N, 9.68; Found: C, 60.92; H, 3.67; N, 9.72.

2-(6-(4-(Trifluoromethyl)phenyl)benzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetic acid (5i)

Yield: 0.49 g (44.5%) as a brown solid; m.p. 262°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.81 (s, 1H), 8.12 (d, ^3J = 7.7 Hz, 1H), 7.70 (d, ^3J = 7.9 Hz, 1H), 7.65 (d, ^3J = 8.1 Hz, 2H), 7.44 (d, ^3J = 8.1 Hz, 2H), 7.41 – 7.34 (m, 3H), 7.22 (t, ^3J = 7.4 Hz, 1H), 7.19 (t, ^3J = 7.3 Hz, 1H), 6.99 (t, ^3J = 7.5 Hz, 1H), 6.85 (d, ^3J = 8.3 Hz, 1H), 4.40 (d, ^2J = 18.3 Hz, 1H), 4.33 (d, ^2J = 18.3 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.0, 146.4, 144.3, 143.3, 142.3, 132.8, 132.4, 129.9 (d, $^2J_{CF}$ = 32.7 Hz), 127.1, 126.3 (d, $^3J_{CF}$ = 3.6 Hz), 125.4, 123.1, 123.0, 119.8, 119.4, 114.8, 114.0, 110.5, 72.2, 51.3; ^{19}F NMR (470 MHz, DMSO-d_6): δ -61.24 (s); MS (CI): *m/z* 424.0 [M + H]$^+$; Anal. Calcd. for $C_{23}H_{16}F_3N_3O_2$: C, 65.25; H, 3.81; N, 9.92; Found: C, 65.20; H, 3.77; N, 9.97.

2-(6-(4-Nitrophenyl)benzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetic acid (5j)

Yield: 0.66 g (63.5%) as a yellow solid; m.p. 246°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.74 (s, 1H), 8.18 – 8.00 (m, 3H), 7.67 (d, ^3J = 7.0 Hz, 1H), 7.45 (d, ^3J = 8.9 Hz, 2H), 7.40 (s, 1H), 7.39 – 7.32 (m, 2H), 7.25 – 7.12 (m, 2H), 6.96 (t, ^3J = 7.5 Hz, 1H), 6.83 (d, ^3J = 8.3 Hz, 1H), 4.39 (d, ^2J = 18.3 Hz, 1H), 4.32 (d, ^2J = 18.3 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.0, 148.2, 146.3, 145.7, 144.3, 142.1, 132.7, 132.5, 127.6, 125.4, 124.6, 123.2, 123.0, 120.0, 119.4, 115.0, 114.0, 110.5, 71.9, 51.4; MS (CI): *m/z* 401.2 [M + H]$^+$; Anal. Calcd. for $C_{22}H_{16}N_4O_4$: C, 66.00; H, 4.03; N, 13.99; Found: C, 66.07; H, 4.08; N, 14.04.

2-(6-(4-Hydroxy-3-methoxyphenyl)benzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetic acid (5k)

Yield: 0.45 g (42.8%) as a light yellow solid; m.p. 209°C; IR: ṽ = 3408 (O-H, str., br.), 3069 (C-H$_{aryl}$, str., m.), 2926 (C-H$_{alkyl}$, str., m.), 1898 (aromatic overtone), 1709 (C=O, str., w.), 1632 – 1513 (C=C$_{aryl}$, str., v.), 1616 (COO$^-$, str., as., s.), 1460 (C-H$_{alkyl}$, bend., m.), 1392 (COO$^-$, str., s., s.), 1288 – 1257 (C-O, str., s.), 1174 – 1028 (C-N, str., v.), 863 (1,2,4-subst. C-H$_{aryl}$, bend., m.), 835 (1,2,4-subst. C-H$_{aryl}$, bend., m.), 742 cm^{-1} (1,2-subst. C-H$_{aryl}$, bend., s.); ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.80 (s, 1H), 9.20 (s, 1H), 8.09 (d, ^3J = 7.4 Hz, 1H), 7.66 (d, ^3J = 7.9 Hz, 1H), 7.36 (t, ^3J = 7.6 Hz, 1H), 7.23 – 7.17 (m, 2H), 7.14 (t, ^3J = 7.5 Hz, 1H), 7.01 – 6.97 (m, 2H), 6.95 (t, ^3J = 7.4 Hz, 1H), 6.80 (d, ^3J = 8.3 Hz, 1H), 6.64 (d, ^3J = 8.2 Hz, 1H), 6.58 (d, ^3J = 8.1 Hz, 1H), 4.22 (d, ^2J = 18.3 Hz, 1H), 4.15 (d, ^2J = 18.2 Hz, 1H), 3.62 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.1, 148.0, 147.9, 146.6, 144.2, 142.9, 133.1, 132.3, 129.5, 125.3, 122.8, 122.6, 119.3, 119.2, 116.1, 113.9, 113.8, 111.2, 111.0, 73.4, 56.0, 50.1; MS (CI): *m/z* 402.2 [M + H]$^+$; Anal. Calcd. for $C_{23}H_{19}N_3O_4$: C, 68.82; H, 4.77; N, 10.47; Found: C, 68.78; H, 4.71; N, 10.52.

2-(6-(Pentafluorophenyl)benzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetic acid (5l)

Yield: 0.34 g (29.3%) as a brown solid; m.p. 259°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.93 (s, 1H), 8.12 (d, ^3J = 7.4 Hz, 1H), 7.80 (s, 1H), 7.70 (d, ^3J = 7.4 Hz, 1H), 7.38 (t, ^3J = 7.6 Hz, 1H), 7.31 – 7.14 (m, 3H), 6.99 (t, ^3J = 7.3 Hz, 1H), 6.79 (d, ^3J = 8.1 Hz, 1H), 4.44 (d, ^2J = 18.8 Hz, 1H), 4.33 (d, ^2J = 18.7 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.1, 146.4, 144.0, 141.9, 132.3, 132.1, 124.9, 123.4, 123.2, 119.6, 119.4, 113.5, 113.2, 109.7, 65.2, 51.1; ^{19}F NMR (470 MHz, DMSO-d_6): δ -142.40 (d, $^3J_{FF}$ = 24.8 Hz), -152.86 (t, $^3J_{FF}$ = 22.3 Hz), -161.52 (td, J_{FF} = 23.7, 7.6 Hz); MS (CI): *m/z* 446.0 [M + H]$^+$; Anal. Calcd. for $C_{22}H_{12}F_5N_3O_2$: C, 59.33; H, 2.72; N, 9.44; Found: C, 59.29; H, 2.77; N, 9.48.

2-(6-(Pyridin-3-yl)benzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetic acid (5m)

Yield: 0.70 g (75.3%) as a light yellow solid; m.p. 274°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.55 (s, 1H), 8.55 (s, 1H), 8.49 – 8.43 (m, 1H), 8.11 (d, ^3J = 7.6 Hz, 1H), 7.70 (d, ^3J = 7.7 Hz, 1H), 7.49 (d, ^3J = 8.1 Hz, 1H), 7.44 (d, ^3J = 7.6 Hz, 1H), 7.39 (t, ^3J = 7.8 Hz, 1H), 7.34 (s, 1H), 7.29 – 7.16 (m, 3H), 7.00 (t, ^3J = 7.5 Hz, 1H), 6.87 (d, ^3J = 8.3 Hz, 1H), 4.41 (d, ^2J = 18.3 Hz, 1H), 4.34 (d, ^2J = 18.2 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.0, 150.8, 147.5, 146.4, 144.3, 142.4, 134.6, 133.9, 132.7, 132.5, 125.4, 124.5, 123.2, 123.0, 120.0, 119.4, 114.9, 114.1, 110.5, 71.0, 51.4; MS (CI): *m/z* 357.2 [M + H]$^+$; Anal. Calcd. for $C_{21}H_{16}N_4O_2$: C, 70.77; H, 4.53; N, 15.72; Found: C, 70.82; H, 4.49; N, 15.78.

2-(6-(5-(Trifluoromethyl)thiophen-2-yl)benzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetic acid (5n)

Yield: 0.74 g (66.1%) as a brown solid; m.p. 252°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.87 (s, 1H), 8.09 (dd, *J* = 7.7, 1.3 Hz, 1H), 7.73 (s, 1H), 7.72 – 7.70 (m, 1H), 7.61 – 7.57 (m, 1H), 7.51 – 7.48 (m, 1H), 7.43 (t, ^3J = 7.0 Hz, 1H), 7.28 – 7.24 (m, 2H), 7.23 – 7.20 (m, 1H), 7.05 (t, ^3J = 7.3 Hz, 1H), 6.96 (d, ^3J = 8.3 Hz, 1H), 4.44 (d, ^2J = 18.2 Hz, 1H), 4.33 (d, ^2J = 18.2 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.9, 146.5, 146.0, 144.2, 141.7, 132.6, 132.5, 130.1 (q, $^3J_{CF}$ = 3.5 Hz), 129.4 (q, $^2J_{CF}$ = 38.5 Hz), 127.0, 125.4, 123.3, 123.2, 122.4 (d, $^1J_{CF}$ = 268.7 Hz), 120.7, 119.5, 115.6, 114.6, 110.5, 68.4, 51.3; ^{19}F NMR (470 MHz, DMSO-d_6): δ -54.19 (s); MS (CI): *m/z* 430.0 [M + H]$^+$; Anal. Calcd. for $C_{21}H_{14}F_3N_3O_2S$: C, 58.74; H, 3.29; N, 9.79; Found: C, 58.68; H, 3.24; N, 9.83.

Potassium 2-(6-(3-methoxy-4-oxidophenyl)benzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetate (6a)

0.1000 g (0.25 mmol) of **5k** and 0.0279 g (0.50 mmol) of KOH in 3 ml of 96% EtOH were stirred for 10 min. The resulting solution was concentrated under reduced pressure at room temperature. The solid residue was thoroughly dried *in vacuo* to give the product as a beige solid. Yield: 0.11 g (92.5%); m.p. 242-247°C; IR: ṽ = 3367 (H_2O), 3066 (C-H_{aryl}, str., m.), 2925 (C-H_{alkyl}, str., m.), 1613 (COO^-, str., as., s.), 1488 (C=C_{aryl}, str., s.), 1450 (C-H_{alkyl}, bend., m.), 1385 (COO^-, str., s., s.), 1301 – 1228 (C-O, str., s.), 1169 – 1028 (C-N, str., v.), 840 (1,2,4-subst. C-H_{aryl}, bend., m.), 820 (1,2,4-subst. C-H_{aryl}, bend., m.), 742 cm^{-1} (1,2-subst. C-H_{aryl}, bend., s.); Anal. Calcd. for $C_{23}H_{17}K_2N_3O_4$: C, 57.84; H, 3.59; N, 8.80; Found: C, 57.80; H, 3.53; N, 8.86.

Potassium 2-(6-(4-methoxyphenyl)benzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetate (6b)

0.1000 g (0.26 mmol) of **5g** and 0.0145 g (0.26 mmol) of KOH in 3 ml of 96% EtOH were stirred for 10 min and worked up as described for **6a** to provide the product as a light green solid. Yield: 0.10 g (91.7%); m.p. 121°C; IR: ṽ = 3368 (H_2O), 3063 (C-H_{aryl}, str., m.), 2930 (C-H_{alkyl}, str., m.), 1610 (COO^-, str., as., s.), 1533 – 1487 (C=C_{aryl}, str., v.), 1450 (C-H_{alkyl}, bend., m.), 1384 (COO^-, str., s., m.), 1303 – 1245 (C-O, str., s.), 1173 – 1028 (C-N, str., v.), 827 (1,4-subst. C-H_{aryl}, bend., m.), 742 cm^{-1} (1,2-subst. C-H_{aryl}, bend., s.); Anal. Calcd. for $C_{23}H_{18}KN_3O_3$: C, 65.23; H, 4.28; N, 9.92; Found: C, 65.29; H, 4.32; N, 9.97.

Biology

Antimicrobial screening

Sterilized filter paper disks (200 g/m^2, 6 mm diameter) impregnated with a solution of the test compound (**5a-n**, **6a**, **6b**) in DMF (1 mg/ml) and dried at 60°C for 1 h were placed on a Mueller-Hinton agar, Mueller-Hinton agar with 5% sheep blood or Sabouraud agar plate seeded with the appropriate test organism. The plates containing bacteria were incubated for 24 h at 37°C, while the one with *C. albicans* for 24 h at 28°C. The utilized test organisms were: *S. aureus* (ATCC 25923) and *S. pyogenes* (the bacterial strain was isolated from the patient's mucous membrane of the pharynx, who was diagnosed with nasopharyngitis) as examples of Gram-positive bacteria, *P. vulgaris* (HX19 №222), *S. typhimurium* (79), *E. coli* (ATCC 25922), *P. aeruginosae* (ATCC 27853), *S. sonnei* (S-form), *K. pneumonia* (K-56 №3534/51) and *K. aerogenes* (NCTC 10006) as examples of Gram-negative bacteria. The test compounds were also evaluated for their *in vitro* antifungal potential against *C. albicans* (ATCC 885-653). Ciprofloxacin was used as a standard for antibacterial assay and ketoconazole – for the antifungal one in the same concentration as for the test compounds. Inhibition zone diameters were measured with a ruler on the undersurface of the Petri dish or with calipers near the agar surface in mm.

Allium test

Equal-sized bulbs of *Allium cepa* L. (4.1-5.9 g) were chosen and placed initially in tap water and incubated at an average temperature of 20°C for 2-3 days. When the roots have reached the length of 0.5-1 cm, the bulbs were transferred to 20 ml test tubes with the test solutions and exposed for 3 days at room temperature. A small amount of each of the above solutions was added to each respective test tube each day in order to replace that lost through evaporation. Roots length was measured to the nearest millimetre using a ruler every day at the same time. Then the rootlets were collected and fixed immediately in Clarke's fluid (EtOH-AcOH 3:1) for 24 h. Afterwards, the rootlets were removed from the fixing solution and transferred to 70% EtOH and stored at 4°C for further experimental work. In the next stage, the root tips were stained with 1% acetocarmine (1.0 g of carmine in 100 ml of 45% AcOH had been refluxed for 3 hours and filtered) with heating on the water bath for 12 min. The root tip was removed from the stain and rinsed in 45% AcOH. The root tip was placed on a microscope slide and 3 mm of the root tip was removed with a scalpel. Two drops of 45% AcOH were added and the root tip was squashed beneath a 40×40 mm cover glass. Observation was done at 600× through the light microscope (MICMED-1 LOMO, objective 40×0.65, eyepiece K15×). The mitotic index was calculated by examining about 600 cells of each root tip. The mitotic index is the percentage of cells in various stages of mitosis relative to the total number of cells examined. A negative control group was treated with tap water and 0.001 N KOH in tap water. Methotrexate hydrate (Sigma-Aldrich) was used as a positive control. The test solutions and a positive control were prepared by the following procedure. In a 50 ml beaker were placed 24 ml of tap water, 1 ml of 0.025 N KOH in tap water (0.05 N KOH for methotrexate hydrate, **5f** and **5k**) and 0.025 mmol of the test

compound (**5a-n**) or methotrexate hydrate with stirring at room temperature. The test compounds (**5a-n**) and methotrexate hydrate were not completely soluble in the alkaline solution, so the slight precipitate was observed at the bottom of the beaker. The resulting liquid was suspended and transferred to the test tube without decantation or filtration.

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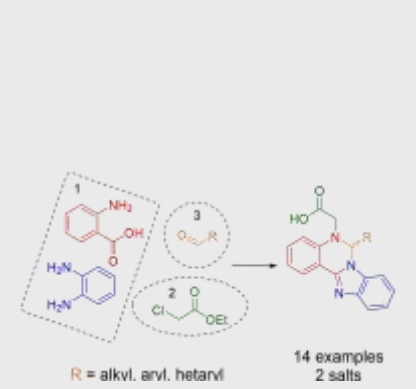

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Synthesis, antimicrobial and mitotic toxicity evaluation of new 6-substituted 2-(benzo[4,5]imidazo[1,2-*c*]quinazolin-5(6*H*)-yl)acetic acids