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Synthesis and characterization of sulphanilamide and benzimidazole pharmacophores containing γ-amino acid derivatives as dual antimicrobial and anticancer agents

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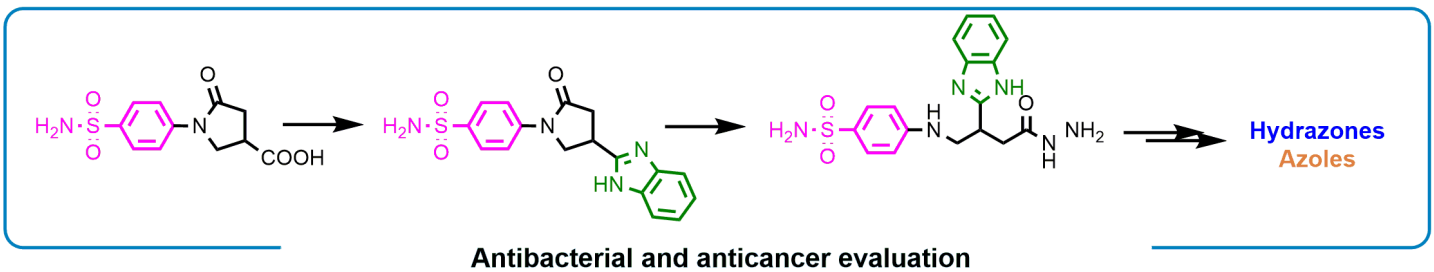
Abstract

A series of γ-amino acid derivatives with sulfanilamide and benzimidazole pharmacophores in the structures were synthesized and evaluated for antibacterial and anticancer activity. Hydrazones with 5-nitrofur-2-yl and 5-nitrothien-2-yl moieties showed good inhibition against *Staphylococcus aureus.* Furthermore, hydrazones with a 5-nitrofur-2-yl moiety and a 2,5-dimethylpyrrole derivative demonstrated excellent inhibition of *Listeria monocytogenes*, which matched to control Cefazolin. Hydrazones bearing a 4-$Me_2NC_6H_4$ fragment had a bactericidal effect against *Escherichia coli* two-fold stronger comparing to control. Hydrazones with 5-nitrofur-2-yl and 5-nitrothien-2-yl substituents were found to be the most active compounds against the Caco-2 colon adenocarcinoma cell line.



Antibacterial and anticancer evaluation

Keywords: Sulfanilamide, benzimidazole, γ-amino acids, hydrazones, azoles, biological activity

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Introduction

Multi-microbial drug resistance is a serious global problem posing a major threat to health worldwide and leading to increased spread of diseases, an exacerbation of serious illnesses and even mortality. The European Centre for Disease Prevention and Control has reported that AMR kills 33,000 people every year in the EU. From the 13.7 million infection-related deaths in 2019 in the world, 7.7 million of them are associated with bacterial pathogens.[1]

Although antibiotics have been one of the greatest success stories in medicine, their improper and excessive use cause major problems that are already difficult to overcome these days. The World Health Organization (WHO) has issued several warnings about the misuse and overuse of antibiotics, which increases antimicrobial resistance,[2] and poses new major global challenges for researchers to find new broad-spectrum antibiotic substances with new mechanisms of action to overcome this problem.

Another huge global problem is the prevalence of various cancers, which are one of the deadliest diseases today and at the same time represent a challenging research task to create new entities that are selective for cancer cells. It is estimated that in 2020 there may have been about 18.1 million cancer cases worldwide.[3] Thus, the prevention of these diseases is one of the most important public health challenges of the 21st century. The emergence of drug resistance and adverse effects of anticancer drugs require increased efforts in the development of new, low-toxicity anticancer drugs with high specificity and efficacy against the target and drug resistance.[4]

Molecules with benzimidazole and sulfanilamide moieties or their hybrid structures are attractive in the search for effective drugs because they quite often exhibit diverse and important biological properties. Variously substituted benzimidazole moieties demonstrate remarkable therapeutic actions including antidiabetic, antihistamine, anti-tuberculosis, anti-malarial, antiviral, anti-inflammatory, analgesic, anti-HIV, proton pump inhibition among others.[5,6] Many attempts have been made to evaluate these molecules for prospective antimicrobial and anticancer activity and this potential has been demonstrated.[7–19] A large number of already developed and approved effective benzimidazole-based anticancer drugs are widely used in clinical therapy.[20,21] The anticancer effect of benzimidazoles occurs through various mechanisms, such as disruption of microtubule polymerization, the induction of apoptosis, cell cycle (G2/M) arrest, antiangiogenesis, and blockage of glucose transport.[22,23]

In medicinal practice, sulfonamides have been widely used for many years, and the interest of substances having the sulfonamide core is constantly growing due to their important therapeutic properties. Studies *in vivo* showed them to demonstrate antiviral,[24] anti-HIV,[25] anti-obesity,[26] antineuropathic,[27] effect and to play an important role in treating various disease states such as diuresis, hypoglycaemia, thyroiditis, inflammation, and glaucoma.[28] Among others, sulfonamides play a prominent role as antibacterials[29] used to treat human and animal bacterial infections and constitutes a considerable library of anticancer agents and drugs with remarkable healing effects against various tumours.[30-34] Their stability, easy preparation and bioavailability explains why this fragment is so widely distributed in medicinal preparations and is further studied in search of new medicinal substances.[28]

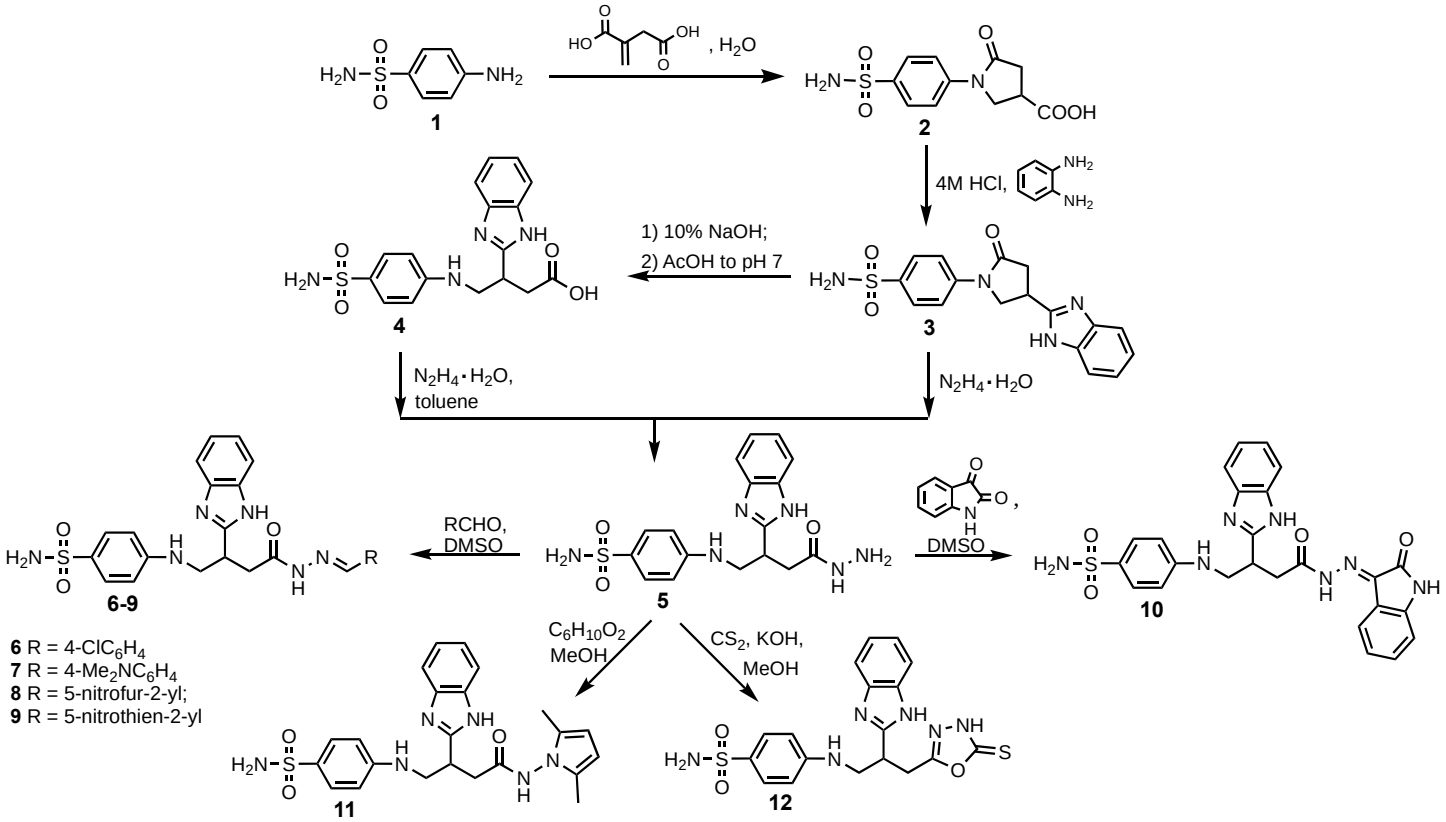
Recent advances in sulfonamide derivatives as potential anticancer agents targeting CA enzymes, antiapoptotic Bcl-2 proteins, aromatase, and others have shown them to be a promising strategy for developing superior anticancer agents by combining a sulfonamide moiety with other pharmacophores in molecules.[34–36] Among them, benzimidazole-anchored sulfonamides should be mentioned which have been reported to possess potential antimicrobial and anticancer properties targeting various resistant microbial species and cancer cells.[37–41]

Encouraged by our previous studies[42–44] and in the knowledge of the previous literature, in the current study we have synthesized and characterized a series of γ-amino acid derivatives having benzimidazole and sulfanilamide cores with the aim of evaluating some of their biological properties.

Results and Discussion

Synthesis

To design new γ-amino acid derivatives, 4-aminobenzenesulfonamide **1** was selected as a parent compound. 5-Oxopyrrolidine-3-carboxylic acid **2** (Scheme 1) was obtained by refluxing aromatic amine **1** with itaconic acid in water[45] producing the compound in a yield of 66%. A previously reported method based on the melting of the reactants produced a lower yield of the target amino acid **2**.[46]



Scheme 1. Synthesis of compounds **2–12**.

To introduce the benzimidazole pharmacophore, acid **2** was reacted with *o*-phenylenediamine in 4M hydrochloric acid. Reflux for 72 hours afforded benzimidazole **3**, which was separated from the reaction mixture by addition of an aqueous 5% sodium carbonate solution. In the ^{1}H NMR spectrum of **3**, a singlet at 7.33 ppm was assigned to the protons of the H_2NSO_2 fragment. The appearance of a singlet at 12.49 ppm confirmed the presence of the NH of benzimidazole structure. Aromatic protons were found to resonate in the range of 7.05–7.90 ppm. From the ^{13}C NMR spectrum, the carbon of the N=C-NH fragment resonated at 154.84 ppm, the C=O spectral line was found at 172.74 ppm and the remaining peaks were in excellent agreement with carbons of the structure **3**. In a previously reported article[46] the benzimidazole **3** was obtained by another

method but in a lower 66% yield.

Since the target hydrazide **5** can be obtained directly from benzimidazole **3** using hydrazine monohydrate or by a two-step reaction, which involve the cleavage of the pyrrolidinone ring in a strongly alkaline environment (aqueous 10% sodium hydroxide) followed by reaction of the resulting γ-amino acid **4** with hydrazine monohydrate in toluene, we assessed tested both methods to obtain higher yields of the target compound. The two-step pathway leading to a 66% yield was clearly superior if compared with the direct method, which gave a 10% lower yield. In the ^{1}H NMR spectrum for compound **5**, a broad singlet at 4.17 ppm, attributed to the protons of the hydrazide NH_2 group, and triplet at 6.55 ppm, attributed to the proton of the NH of the $NHNH_2$ group, prove the presence of the hydrazide moiety. The observed resonance peak at 169.85 ppm in the ^{13}C NMR indicates the formation of an open-chain structure with the $CONHNH_2$ moiety if compared to the structure of compound **3**, in which spectrum the carbonyl carbon resonates at the characteristic position of the C-2 of a pyrrolidin-2-one ring, i.e. at 172.74 ppm.

According to the literature, hydrazones having 4-ClC_6H_4- and 4-$Me_2NC_6H_4$- substituents as well as 5-nitrofur-2-yl or 5-nitrothien-2-yl substitutions demonstrate significant antibacterial properties.[47] The nitro substituent is also known as important pharmacophore present in the structures of antibacterial substances.[48] Accordingly, we synthesized hydrazones with the above-mentioned molecular moieties to ascertain their influence on the bioactivity. For this purpose, the reactions of hydrazide **5** with the aldehydes were carried out in DMSO, due to the poor solubility of **5** in other solvents. The products were isolated from the reaction mixtures by pouring them into ice-water mixtures.

By detailed theoretical and experimental study,[49,50] the presence of the amide (CO–NH) bond and the restricted rotation around it causes the formation of conformational isomers. Thus, hydrazones in DMSO solutions exist as a mixture of the *Z* and *E* rotamers in which the *Z* structure predominates. According to this, the protons of the CONH and N=CH fragments are observed in two sets of resonances in the ^{1}H NMR spectra recorded in DMSO-d_6, where a stronger-field side peak is related to the *Z*-form isomer. In the ^{1}HNMR spectra of hydrazones **6–9**, the NH singlets resonated between 11.01 and 11.94 ppm as two-sets of peaks. From the ^{1}H NMR spectral data, the calculated isomer molar ratio was found of 60: 40 in all cases.

According to the literature, hydrazones can exist in a mixture of geometric isomers due to the presence of an azomethine group in their structure.[51–53] Research has shown that hydrazones derived from arylaldehydes exist in the *E* configuration at the C=N double bond.[51–53] The spectral data of the resulting hydrazones **6–9** confirmed the presence of only one geometric isomer, which was therefore assigned the *E* form.

The search of isatin-based compounds with biological activity often attracts the attention of chemists. As has been reported, in recent years, the screening of various derivatives based on the 2-oxoindolin-3-ylidene core for the antibacterial efficacy[54] and anticancer properties[55,56] showed them to possess very promising potency. To verify the effect of this fragment on the bio properties of the target derivatives, we synthesized 4-((2-(1*H*-benzo[d]imidazol-2-yl)-4-oxo-4-(2-(2-oxoindolin-3-ylidene)hydrazinyl)butyl)amino)-benzenesulfonamide **10** based on the hydrazide **5** skeleton containing a 2-oxoindolin-3-ylidene nucleus. This was realized by treating of compound **5** with isatin in dimethylsulfoxide at 80 °C for 7 hours. The product **10** was isolated from the reaction mixture as for hydrazones **6–9**.

The ^{1}H NMR spectrum of isatin derivative **10** displayed a multiplet in the range of 3.36–3.88 ppm for alkyl protons, the secondary amine group showed a multiplet in the interval of 6.54–6.71 ppm, and the benzimidazole NH proton was observed at the characteristic region, namely at 12.40 ppm. As is known, the hydrazones may exist as *Z/E* geometrical isomers around C=N double bond and as *cis/trans* amide conformers.[56,57] However, the ^{1}H NMR spectrum of **10** demonstrated only the presence of the *E* geometrical isomer. Two sets of protons of the CONH group (^{1}H NMR, 11.18 and 12.52 ppm, intensity ratio 70: 30) indicated

a mixture of conformers due to the restricted rotation around the amide bond. The NH proton of the isatin fragment was found to resonate at 10.81 ppm.

Next, hydrazide **5** was applied for the preparation of heterocyclic products **11** and **12** to determine their biological efficacy, because it is well known that modification of a parent molecule with pyrrole and 1,3,4-oxadiazolthione substitutions increases the biological properties of the compound.[55,58,59] As expected, hydrazide **5**, when derivatized with hexane-2,5-dione in methanol at reflux for 2 hours, with the presence of a catalytic amount of glacial acetic acid, afforded 3-(1*H*-benzo[*d*]imidazol-2-yl)-*N*-(2,5-dimethyl-1*H*-pyrrol-1-yl)-4-((4-sulfamoylphenyl)amino)butanamide **(11)**. Singlets at 1.59 and 1.93 (CH_3) and at 5.51 and 5.56 (CH-CH) ppm in the ^{1}H NMR spectrum, and resonances at 10.48, 11.00, and 102.77, 102.84 ppm of the appropriate fragments in the ^{13}C NMR spectrum, confirmed the presence of the 2,5-dimethylpyrrole ring.

For the preparation of the 1,3,4-oxadiazole derivative **12**, the hydrazide **5** was refluxed with carbon disulfide in a methanolic solution of potassium hydroxide. To isolate the target product **12**, the aqueous solution of the residue of the reaction mixture was acidified with aqueous 15% hydrochloric acid to pH 5. The ^{13}C NMR spectrum fully proved the presence of the 1,3,4-oxadiazole thione structure, demonstrating characteristic resonances at 162.05 and 177.58 ppm, corresponding to C-2 and C-5 of the newly formed heterocycle. A broad singlet at 14.13 ppm integrated for two protons was assigned to the NH groups of both benzimidazole and oxadiazole rings. Thus, the structures of the target products were established based on the NMR, IR and microanalysis data.

Antimicrobiobial and anticancer properties

The *in vitro* antimicrobial properties of the eleven synthesized compounds were investigated. Gram-positive and Gram-negative bacteria were used in the study. *Staphylococcus aureus* subsp. *aureus* (ATCC 9144) and zoonotic agent *Listeria monocytogenes* (ATCC 35152) represented Gram-positive bacteria, while Gram-negative ones were represented by *Escherichia coli* (ATCC 13076), and zoonotic agent *Salmonella enterica* subsp. *enterica* serovar Enteritidis (ATCC 8739). The antibiotic cefazolin was used as a standard in the antibacterial tests.

The results showed that hydrazone **7** inhibited gram-negative *E. coli* most effectively, with an MIC of 15.6 μg/mL. Hydrazones **8** and **9** having 5-nitrofur-2-yl and 5-nitrothien-2-yl moieties, respectively showed the highest antibacterial efficacy against Gram-positive *S. aureus* with MIC of 31.3 *μ*g/mL and MBC of 62.5 *μ*g/mL while hydrazone **8** and pyrrole **11** appeared to be the strongest against Gram-positive *L. monocytogenes* with MIC of 31.3 *μ*g/mL and MBC of 62.5 *μ*g/mL (Supplementary Materials).

To evaluate the anticancer properties of the synthesized compounds we employed human lung and colonic adenocarcinoma models (A549 and Caco-2) *in vitro* and compared them with doxorubicin (DOX) and cisplatin (CP) which are approved anticancer drugs used for treatment lung and colon cancers. Results demonstrated that hydrazone **8** with 5-nitrofuranyl fragment had strong anticancer activity against Caco-2 cells (33.7%) in comparison with control (p<0.05). Similar results were obtained with hydrazone **9** with a 5-nitro-2-thienyl group moiety in the structure. This compound also significantly reduced Caco-2 viability (to 45.5%) in comparison with the control drug (p<0.05), suggesting that the 5-nitrofur-2-yl and 5-nitrothien-2-yl substituents in the hydrazone structure are essential for selective anticancer properties targeting Caco-2 colorectal adenocarcinoma cells (Supplementary Materials).

Conclusions

Some sulfanilamide/benzimidazole-based γ-amino acid derivatives as potential antibacterial and anticancer substances were designed and synthesized. Their MIC and MBC were evaluated by both microdilution and growth on agar techniques. The antibacterial susceptibility test confirmed that all tested derivatives possess an inhibitory effect on the growth of both Gram-positive and Gram-negative bacteria. However, the compounds showed the potency of antibacterial efficacy at lower or higher concentrations for all or some strains of bacteria. Higher concentrations were required to inhibit and kill *Salmonella* Enteritidis in the most cases. The compounds showed the higher range of MIC and MBC against Gram-negative *E. coli* and Gram-positive *S. aureus* and *L. monocytogenes*. 4-((2-(1*H*-benzo[d]imidazol-2-yl)-4-(2-(4-(dimethylamino)benzylidene)hydrazinyl)-4-oxobutyl)amino)benzenesulfonamide **(7)** showed the highest antibacterial efficacy against Gram-negative *E. coli* with MIC of 15.6 μg/mL and MBC of 62.5 μg/mL while the hydrazones with 5-nitrofur-2-yl **(8)** and 5-nitrothien-2-yl **(9)** moieties appeared the strongest against Gram-positive *S. aureus* with MIC of 31.3 μg/mL and MBC of 62.5 μg/mL. Furthermore, hydrazone **8** and pyrrole derivative **9** revealed the strongest antibacterial properties against Gram-positive *L. monocytogenes* with MIC of 31.3 μg/mL and MBC of 62.5 μg/mL.

As for anticancer activity, 4-((2-(1*H*-benzo[*d*]imidazol-2-yl)-4-(2-((5-nitrofuran-2-yl)methylene)hydrazinyl)-4-oxobutyl)amino)- **(8)** and 4-((2-(1*H*-benzo[*d*]imidazol-2-yl)-4-(2-((5-nitrothiophen-2-yl)methylene)hydrazinyl)-4-oxobutyl)amino)benzenesulfonamides **(9)**, which selectively reduced cell viability at 100 µM concentration up to 33.7 and 45.5%, respectively, have been identified as the most active compounds against human Caco-2 colon adenocarcinoma cell line.

Experimental Section

General. Reagents, antibiotics, and solvents were obtained from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification. The reaction course and purity of the synthesized compounds were monitored by TLC using aluminium plates precoated with silica gel with F254 nm (Merck KGaA, Darmstadt, Germany). Melting points were determined with a B-540 melting point analyser (Büchi Corporation, New Castle, DE, USA) and were uncorrected. NMR spectra were recorded on a Brucker Avance III (400 MHz, for ^{1}H NMR, 101 MHz for ^{13}C NMR) spectrometer (Bruker BioSpin AG, Fällanden, Switzerland). Chemical shifts were reported in (d) ppm relative to tetramethylsilane (TMS) with the residual solvent as internal reference (DMSO-d_6, δ = 2.50 ppm for ^{1}H and δ = 39.5 ppm for ^{13}C). Data were reported as follows: chemical shift, multiplicity, coupling constant (Hz), integration, and assignment. IR spectra (v, cm^{-1}) were recorded on a Perkin–Elmer Spectrum BX FT–IR spectrometer (Perkin–Elmer Inc., Waltham, MA, USA) using KBr pellets. Elemental analyses (C, H, N) were conducted using the Elemental Analyzer CE-440 (Exeter Analytical, Inc., Chelmsford, MA, USA); their results were found to be in good agreement (±0.4%) with the calculated values.

5-Oxo-1-(4-sulfamoylphenyl)pyrrolidine-3-carboxylic acid (2). Prepared from 4-aminobenzenesulfonamide **(1)** (0.175 mol, 30.14 g) and itaconic acid according to the method described in literature.[45] Yield 33.28 g (66%), mp 199–200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.68–2.90 (m, 2H, CH_2CO), 3.35–3.41 (m, 1H, CH), 3.96–4.16 (m, 2H, NCH_2), 7.31 (s, 2H, NH_2), 7.81 (d, *J* 9.0 Hz, 2H, H_{ar}), 7.85 (d, *J* 9.0 Hz, 2H, H_{ar}), 12.72 (br. s, 1H, OH) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ: 35.10, 35.32 ($\underline{CH_2}CO$, CH), 49.93 (NCH_2), 118.91, 126.50, 139.03, 141.82 (C_{ar}), 172.59 (CO), 174.10 (COOH) ppm. IR, v: 3317 (NH_2), 3208 (OH), 1711, 1687 (2C=O), 1163 (S=O) cm.$^{-1}$Calcd. for $C_{11}H_{12}N_2O_5S$, %: C 46.47; H 4.25; N 9.85. Found, %: C 46.54; H 4.11; N 9.62.

4-(4-(1*H*-Benzo[*d*]imidazol-2-yl)-2-oxopyrrolidin-1-yl)benzenesulfonamide (3). A mixture of carboxylic acid **2** (0.107 mol, 30.42 g), benzene-1,2-diamine (0.128 mol, 13.88 g) and 4M hydrochloric acid (110 mL) was refluxed for 72 h, then neutralized with aqueous 5% sodium carbonate (400 mL). The solution was boiled, then cooled and the liquid phase was poured off. The residue in the flask was mixed with water (300 mL), then the obtained mixture was boiled and left to cool. After the mixture had settled, the liquid phase was poured off and the residue was left to cool in refrigerator. After cooling, the formed crystalline solid was filtered off, washed with water and dried. Yield 34.43 g (90%), mp 281–282 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.95–3.20 (m, 2H, CH_2CO), 4.04 (p, *J* 7.5 Hz, 1H, CH), 4.10–4.53 (m, 2H, NCH_2), 7.05–7.25 (m, 2H, H_{benz}), 7.33 (s, 2H, NH_2), 7.53 (br. s, 2H, H_{benz}), 7.83 (d, *J* 8.5 Hz, 2H, H_{ar}), 7.89 (d, *J* 8.5 Hz, 2H, H_{ar}), 12.49 (s, 1H, NH) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ: 30.57, 37.67 ($\underline{C}H_2CO$, CH), 52.09 ($NH\underline{C}H_2$), 111.04, 111.38, 118.93, 121.62, 121.72, 126.52, 139.02, 141.92 (C_{ar}), 154.84 (N=C-NH), 172.74 (CO) ppm. IR, v: 3312, 3276 (NH_2), 3155 (NH), 1699 (CO), 1597 (C=N), 1164 (S=O) cm^{-1} Calcd. for $C_{17}H_{16}N_4O_3S$, %: C 57.29; H 4.52; N 15.72. Found, %: C 57.18; H 4.48; N 15.79.

3-(1*H*-Benzo[*d*]imidazol-2-yl)-4-((4-sulfamoylphenyl)amino)butanoic acid (4). A mixture of benzimidazole **3** (0.09 mol, 32.08 g) and aqueous 10% NaOH solution (150 mL), was heated at reflux for 20 h, then filtered off, and the filtrate was neutralized with acetic acid to pH 6–7. The formed precipitate was filtered off, washed with water and purified by dissolving it in aqueous 10% NaOH solution (100 mL), filtering and acidifying the filtrate with acetic acid to pH 6–7. Yield 29.47 g (87%), mp 212–213 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.70–2.96 (m, 2H, CH_2CO), 3.35–3.65 (m, 3H, CH, $NHC\underline{H_2}$), 6.60 (t, *J* 6.2 Hz, 1H, $N\underline{H}CH_2$), 6.71 (d, *J* 8.5 Hz, 2H, H_{ar}), 6.94 (s, 2H, NH_2), 7.13 (dd, *J* 6.1, 3.2 Hz, 2H, H_{ar}), 7.26–7.80 (m, 4H, H_{ar}), 12.28 (br. s, 1H, NH) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ: 35.32, 35.80 ($\underline{CH_2}CO$, CH), 46.26 ($NHCH_2$), 110.94, 121.30, 127.41, 130.38, 151.02, 155.68 (C_{ar}), 173.07 (CO) ppm. IR, v: 3395, 3274, 3171 (NH_2, NH, OH), 1599 (CO), 1553 (C=N), 1149 (SO) cm^{-1}. Calcd. for $C_{17}H_{18}N_4O_4S$, %: C 54.53; H 4.85; N 14.96. Found, %: C 54.47; H 4.83; N 15.10.

4-((2-(1*H*-Benzo[*d*]imidazol-2-yl)-4-hydrazinyl-4-oxobutyl)amino)benzenesulfonamide (5)

Method A. A mixture of compound **3** (0.066 mol, 2.35 g) and hydrazine monohydrate (0.35 mol, 17 mL) was refluxed for 2 h, and then cooled. The obtained crystalline solid was filtered off, washed of Et_2O and recrystalized from MeOH. Yield 1.44 g (56%).

Method B. To a solution of acid **4** (0.01 mol. 3.74 g) in toluene (20 mL), hydrazine monohydrate was added dropwise (0.03 mol, 1.46 mL) and the mixture was heated at reflux for 7 h, then cooled, toluene was decanted off, and the residue was dissolved in MeOH by boiling, filtered and left to cool. The formed solid was filtered off, washed with MeOH, cold Et_2O and dried. Yield 2.58 g (66%), mp 227–228 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.53–2.69 (m, 2H, CH_2CO), 3.38–3.54 (m, 2H, $NHC\underline{H_2}$), 3.70 (p, *J* 6.7 Hz, 1H, CH), 4.17 (br. s, 2H, $NHN\underline{H_2}$), 6.55 (t, *J* 6.1 Hz, $N\underline{H}CH_2$), 6.69 (d, *J* 8.5 Hz, 2H, H_{ar}), 6.93 (s, 2H, NH_2), 7.12 (dd, *J* 6.1, 3.2 Hz, 2H, H_{benz}), 7.41–7.49 (m, 2H, H_{benz}), 7.50 (d, *J* 8.5 Hz, 2H, H_{ar}), 9.09 (s, 1H, $\underline{NH}NH_2$), 12.27 (br. s, 1H, NH) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ: (400 MHz, DMSO-d_6) δ: 35.29, 35.65 ($\underline{CH_2}CO$, CH), 46.24 ($NHCH_2$), 110.92, 118.30, 121.15, 127.38, 130.26, 133.69, 143.25, 151.10, 155.99 (C_{ar}), 169.85 (CO) ppm. IR, v: 3320, 3230, 3153 (NH_2, NH), 1666 (C=O), 1603 (C=N), 1146 (S=O) cm^{-1}. Calcd. for $C_{17}H_{20}N_6O_3S$, %: C 52.56; H 5.19; N 21.64. Found, %: C 52.49; H 5.22; N 21.59.

General procedure for the preparation of hydrazones 6–9

To a solution of hydrazide **5** (1 mmol, 0.407 g) in DMSO (6 mL) the corresponding aldehyde was added (1.5 mmol) and the mixture was stirred at 130 °C for 3.5 h. After completion, the mixture was cooled and poured over ice-water mixture (10 mL), slowly stirred for 15 min and left in refrigerator overnight. The formed crystalline solid was filtered off, washed with water and recrystalized from MeOH.

4-((2-(1*H*-Benzo[*d*]imidazol-2-yl)-4-(2-(4-chlorobenzylidene)hydrazinyl)-4-oxobutyl)amino)benzenesulfonamide (6). Yield 0.19 g (56%), mp 152–153 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: (*Z/E* 60:40) 2.66–3.30 (m, 2H, CH_2CO), 3.40–3.85 (m, 3H, CH, $NHC\underline{H_2}$), 6.45–6.84 (m, 3H, $N\underline{H}CH_2$, H_{ar}), 6.94 (s,

2H, NH_2), 7.03–7.23 (m, 2H, H_{ar}), 7.35–7.61 (m, 6H, H_{ar}), 7.67 (t, *J* 7.9 Hz, 3H, H_{ar}), 7.96, 8.11 (2s, 1H, N=CH), 11.36, 11.59 (2s, 1H, CONH), 12.35 (s, 1H, NH) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ: 34.48, 34.63, 35.17, 36.17 ($\underline{CH_2}CO$, CH), 46.39, 46.44 ($NHCH_2$), 110.93, 118.19, 121.11, 127.38, 128.37, 128.61, 128.85, 128.90, 129.12, 130.05, 130.36, 133.16, 134.17, 141.64, 144.66, 151.09, 155.74, 156.09, 160.64, 167.16 (C_{ar}, N=CH), 172.68 (CO) ppm. IR, v: 3409, 3308, 3200 (NH, NH_2), 1649 (C=O), 1598 (C=N), 1153 (S=O) cm^{-1}. Calcd. for $C_{24}H_{23}ClN_6O_3S$, %: C 56.41; H 4.54; N 16.45. Found, %: C 56.49; H 4.60; N 16.49.

4-((2-(1*H*-Benzo[*d*]imidazol-2-yl)-4-(2-(4-(dimethylamino)benzylidene)hydrazinyl)-4-oxobutyl)amino)benzenesulfonamide (7). Yield 0.23 g (65%), mp 221–222 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: (*Z/E* 60:40) 2.67–2.87 (m, 1H, CH_2CO), 2.94, 2.96, 2.98 (3s, 6H, $N(CH_3)_2$), 3.10–3.27 (m, 1H, CH_2CO), 3.43–3.51 (m, 1H, $NHC\underline{H_2}$), 3.56–3.65 (m, 1H, $NHC\underline{H_2}$), 3.73–3,86 (m, 1H, CH), 6.57–6.78 (m, 5H, H_{ar}, $N\underline{H}CH_2$), 6.93, 6.95 (2s, 2H, NH_2), 7.13 (br. s, 2H, H_{ar}), 7.40–7.50 (m, 4H, H_{ar}), 7.53 (d, *J* 8.4 Hz, 2H, H_{ar}), 7.85, 7.98 (2s, 1H, N=CH), 11.01, 11.21 (2s, 1H, CONH), 12.34 (s, 1H, NH) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ: 34.71, 35.27, 36.29 ($\underline{CH_2}CO$, CH), 39.81 ($N(CH_3)_2$), 46.37, 46.54 ($NHCH_2$), 110.93, 111.77, 111.84, 121.27, 121.63, 127.40, 128.03, 128.32, 129.53, 130.24, 130.33, 143.80, 146.84, 151.10, 151.14, 151.31, 151.42, 155.89, 156.27, 159.86, 166.38 (C_{ar}, N=CH), 172.00 (CO) ppm. IR, v: 3430, 3314, 3214 (NH, NH_2), 1642 (C=O), 1599 (C=N), 1160 (S=O) cm^{-1}. Calcd. for $C_{26}H_{29}N_7O_3S$, %: C 60.10; H 5.63; N 18.87. Found, %: C 60.15; H 5.59; N 18.80.

4-((2-(1*H*-Benzo[*d*]imidazol-2-yl)-4-(2-((5-nitrofuran-2-yl)methylene)hydrazinyl)-4-oxobutyl)amino)benzenesulfonamide (8). Yield 0.41 g (79%), mp 158–159 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: (*Z/E* 60:40) 2.75–3.18 (m, 1.6H, CH_2CO), 3.39–3.47 (m, 1.4H, $NHC\underline{H_2}$, CH_2CO), 3.53–3.65 (m, 1H, $NHC\underline{H_2}$), 3.71–3.85 (m, 1H, CH), 6.52–6.90 (m, 3H, H_{ar} $N\underline{H}CH_2$), 6.94 (s, 2H, NH_2), 7.03–7.26 (m, 3H, H_{ar}), 7.38–7.61 (m, 4H, H_{ar}), 7.74–7.83 (m, 1H, H_{ar}), 7.91, 8.09 (2s, 1H, N=CH), 11.70, 11.94 (2s, 1H, NHCO), 12.42 (br. s, 1H, NH) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ: 34.13, 34.31, 35.13, 36.18 ($\underline{CH_2}CO$, CH), 46.35, 46.36 ($NHCH_2$), 110.95, 114.79, 121.37, 127.39, 130.35, 130.36, 131.21, 131.22, 133.92, 150.99, 151.66, 151.76 (C_{ar}, NCH), 173.02 (CO) ppm. IR, v: 3373, 3262 (NH, NH_2), 1681 (C=O), 1599 (C=N), 1151 (S=O) cm^{-1}. Calcd. for $C_{22}H_{21}N_7O_6S$, %: C 51.66; H 4.14; N 19.17. Found, %: C 51.60; H 4.13; N 19.22.

4-((2-(1*H*-Benzo[*d*]imidazol-2-yl)-4-(2-((5-nitrothiophen-2-yl)methylene)hydrazinyl)-4-oxobutyl)amino)benzenesulfonamide (9). Yield 0.39 g (72%), mp 161–162 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: (*Z/E* 60:40) 2.80–3.17 (m, 1.4H, CH_2CO), 3.3–3.40 (m, 0.6H, CH_2CO), 3.42–3.62 (m, 2H, $NHC\underline{H_2}$), 3.72–3.84 (m, 1H, CH), 6.56–6.69 (m, 1H, $N\underline{H}CH_2$), 6.74 (t, *J* 8.1 Hz, 2H, H_{ar}), 6.93, 6.95 (2s, 2H, NH_2), 7.07–7.17 (m, 2H, H_{ar}), 8.06–8.22 (m, 2H, H_{ar}), 8.37, 8.62 (2s, 1H, N=CH), 11.71, 11.92 (2s, 1H, NHCO), 12.36 (s, 1H, NH) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ: 34.10, 34.54, 35.15, 36.17 ($\underline{CH_2}CO$, CH), 46.41, 46.43 ($NHCH_2$), 110.96, 118.29, 120.94, 121.52, 127.40, 128.91, 129.49, 130.32, 130.59, 133.48, 136.28, 139.58, 144.19, 146.65, 150.41, 151.04, 155.99, 157.34, 167.66 (C_{ar}, N=CH), 172.91 (CO) ppm. IR, v: 3393, 3276 (NH, NH_2), 1694 (C=O), 1600 (C=N), 1149 (S=O) cm^{-1}. Calcd. for $C_{22}H_{21}N_7O_5S_2$, %: C 50.08; H 4.01; N 18.58. Found, %: C 50.13; H 3.97; N 18.51.

4-((2-(1*H*-Benzo[*d*]imidazol-2-yl)-4-oxo-4-(2-(2-oxoindolin-3-ylidene)hydrazinyl)butyl)amino)benzenesulfonamide (10). To a solution of hydrazide **5** (0.77 mmol, 0.3 g) in DMSO (5 mL) isatin (0.77 mmol, 0.144 g) was added and the mixture was stirred at 80 °C for 7 h. Then the mixture was poured over ice-water mixture and left to crystallize. The obtained precipitate was filtered off, washed with water and recrystallized from propan-2-ol. Yield 0.34 g (85%), mp 195–196 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.36–3.63 (m, 4H, CH_2CO, $NHC\underline{H_2}$), 3.75–3.88 (m, 1H, CH), 6.54–6.71 (m, 1H, $N\underline{H}CH_2$), 6.76 (d, *J* 8.5 Hz, 2H, H_{ar}), 6.89 (d, *J* 7.8 Hz, 1H, H_{ar}), 6.94 (s, 2H, NH_2), 7.01 (t, *J* 7.6 Hz, 1H, H_{ar}), 7.07–7.18 (m, 2H, H_{benz}), 7.37 (t, *J* 7.6 Hz, 1H, H_{ar}), 7.39–7.51 (m, 2H, H_{ar}), 7.53 (d, *J* = 8.5 Hz, 2H, H_{ar}), 8.05 (br. s, 1H, H_{ar}), 10.81 (s, 1H, NH_{isat}), 11.18 (s, 0.7H, NHCO), 12.40 (s, 1H, NH_{benz}), 12.52 (s, 0.3H, NHCO) ppm. IR, v: 3350, 3260 (NH, NH_2), 1722, 1693

(2C=O), 1599 (C=N), 1151 (S=O) cm^{-1}. Calcd. for $C_{25}H_{23}N_7O_4S$, %: C 58.02; H 4.48; N 18.94. Found, %: C 57.97; H 4.48; N 19.01.

3-(1*H*-Benzo[*d*]imidazol-2-yl)-*N*-(2,5-dimethyl-1*H*-pyrrol-1-yl)-4-((4-sulfamoylphenyl)amino)butanamide (11). To a solution of hydrazide **5** (1.3 mmol, 0.5 g) in MeOH (15 mL) hexane-2,5-dione (1.95 mmol, 0.23 mL) was added dropwise, followed by the addition of a few drops of glacial acetic acid. The mixture was refluxed for 2 h. The product precipitated already during the reaction. After completion, the hot mixture was filtered, the crystalline solid was washed with MeOH. To separate the dissolved product, the filtrate was left to cool. The collected product was recrystalized from MeOH. Yield 0.35 g (58%), mp 258–259 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.59, 1.94 (2s, 6H, $2CH_3$), 2.80–2.99 (m, 2H, CH_2CO), 3.43–3.49 (m, 1H, $NHCH_2$), 3.58–3.68 (m, 1H, $NHCH_2$), 3.72–3.79 (m, 1H, CH), 5.51 (d, *J* 3.5 Hz, 1H, CH), 5.57 (d, *J* 3.5 Hz, 1H, CH), 6.66 (t, *J* 6.1 Hz, 1H, $NHCH_2$), 6.73 (d, *J* 8.4 Hz, 2H, H_{ar}), 6.95 (s, 2H, NH_2), 7.14 (dd, *J* = 6.0, 3.2 Hz, 2H, H_{ar}), 7.45–7.52 (m, 2H, H_{ar}), 7.53 (d, *J* = 8.4 Hz, 2H, H_{ar}), 10.73 (s, 1H, CONH), 12.36 (br. s, 1H, NH) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ: 10.48, 11.00 ($2CH_3$), 35.29, 35.49 (CH_2CO, CH), 46.36 ($NHCH_2$), 102.77, 102.84 ($2CH_{pyrrol}$), 110.96, 118.27, 121.23, 126.59, 126.89, 127.43, 130.44, 134.20, 143.26, 151.05, 155.23 (C_{ar}, C_{pyrrol}), 170.19 (CO) ppm. IR, v: 3408, 3372, 3266, 3176 ($NHNH_2$), 1662 (C=O), 1599 (C=N), 1160 (S=O) cm^{-1}. Calcd. for $C_{23}H_{26}N_6O_3S$, %: C 59.21; H 5.62; N 18.01. Found, %: C 58.95; H 5.70; N 17.97.

4-((2-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)propyl)amino)benzenesulfonamide (12). To a mixture of potassium hydroxide (4.5 mmol, 0.25 g) and MeOH (15 mL) carbon disulfide (3.9 mmol, 0.23 mL) was added dropwise and the mixture was stirred at rt for 20 min. Afterwards, the solution of hydrazide **5** (1.3 mmol, 0.5 g) in MeOH (100 mL) was slowly poured into the alcoholic solution and then the mixture refluxed for 8 h. After completion, MeOH was evaporated under reduced pressure, the residue was dissolved in water and acidified with aqueous 15% hydrochloric acid solution to pH 5. The obtained precipitate was filtered off, washed with water and recrystallized from propan-2-ol. Yield 0.46 g (81%), mp 192–193 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.25–3.53 (m, 2H, CH_2C), 3.53–3.94 (m, 3H, $NHCH_2$, CH), 6.72 (d, *J* 8.6 Hz, 2H, H_{ar}), 6.96 (s, 2H, NH_2), 7.19–7.43 (m, 2H, H_{benz}), 7.53 (d, *J* 8.6 Hz, 2H, H_{ar}), 7.56–7.71 (m, 2H, H_{benz}), 14.13 (br. s, 2H, 2NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 26.96, 35.60 (CH_2CO, CH), 45.78 ($NHCH_2$), 111.08, 114.18, 114.51, 123.07, 123.09, 127.41, 130.82, 150.62, 153.71, 162.05 (C_{ar}), 177.58 (CO). IR, v: 3353, 3259 (NH, NH_2), 1625, 1599 (C=N), 1274 (C=S), 1150 (S=O) cm^{-1}. Calcd. for $C_{18}H_{18}N_6O_3S_2$, %: C 50.22; H 4.21; N 19.52. Found, %: C 50.29; H 4.18; N 19.56.

Supplementary Material

^{1}H and ^{13}C NMR spectroscopic data for the synthesized compounds and bioassay protocols and data can be found in the Supplementary Material file.

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