HETEROCYCLES, Vol. 92, No. 2, 2016, pp. 235 - 251. © 2016 The Japan Institute of Heterocyclic Chemistry
Received, 16th October, 2015, Accepted, 6th December, 2015, Published online, 15th January, 2016
DOI: 10.3987/COM-15-13343

SYNTHESIS OF NOVEL BENZIMIDAZOLES 2-FUNCTIONALIZED WITH PYRROLIDINONE AND γ-AMINO ACID WITH A HIGH ANTIBACTERIAL ACTIVITY

Vestina Strelciunaite,[1] Kazimieras Anusevicius,[1] Ingrida Tumosiene,[1] Jurate Siugzdaite,[2] Ilona Jonuskiene,[1] Irena Ramanauskaite,[1] and Vytautas Mickevicius[1]

[1]Department of Organic Chemistry, Kaunas University of Technology, Radvilenu rd. 19, LT-50254 Kaunas, Lithuania; E-mail: ilona.jonuskiene@ktu.lt
[2]Department of Infectious Diseases, Veterinary Academy, Lithuanian University of Health Sciences, Tilzės str. 18, LT-47181 Kaunas, Lithuania

Abstract – Series of 2- and 1,2-disubstituted benzimidazoles with carboxyalkyl substituents or their derivatives were synthesized during chemical transformations of 4-(1*H*-benzimidazol-2-yl)-1-(3-chloro-4-methoxyphenyl)pyrrolidin-2-one. Condensation products of 2-{2-[1-(3-chloro-4-metoxylphenyl)-5-oxo-3-pyrrolidinyl]-1*H*-benzimidazol-1-yl}acetohydrazide and 3-(1*H*-benzimidazol-2-yl)-4-(3-chloro-4-methoxyphenylamino)butanoic acid hydrazide with mono- and dicarbonylic compounds have been obtained. Some of the synthesized compounds are characterized by a strong bactericidal effect, even at low concentrations.

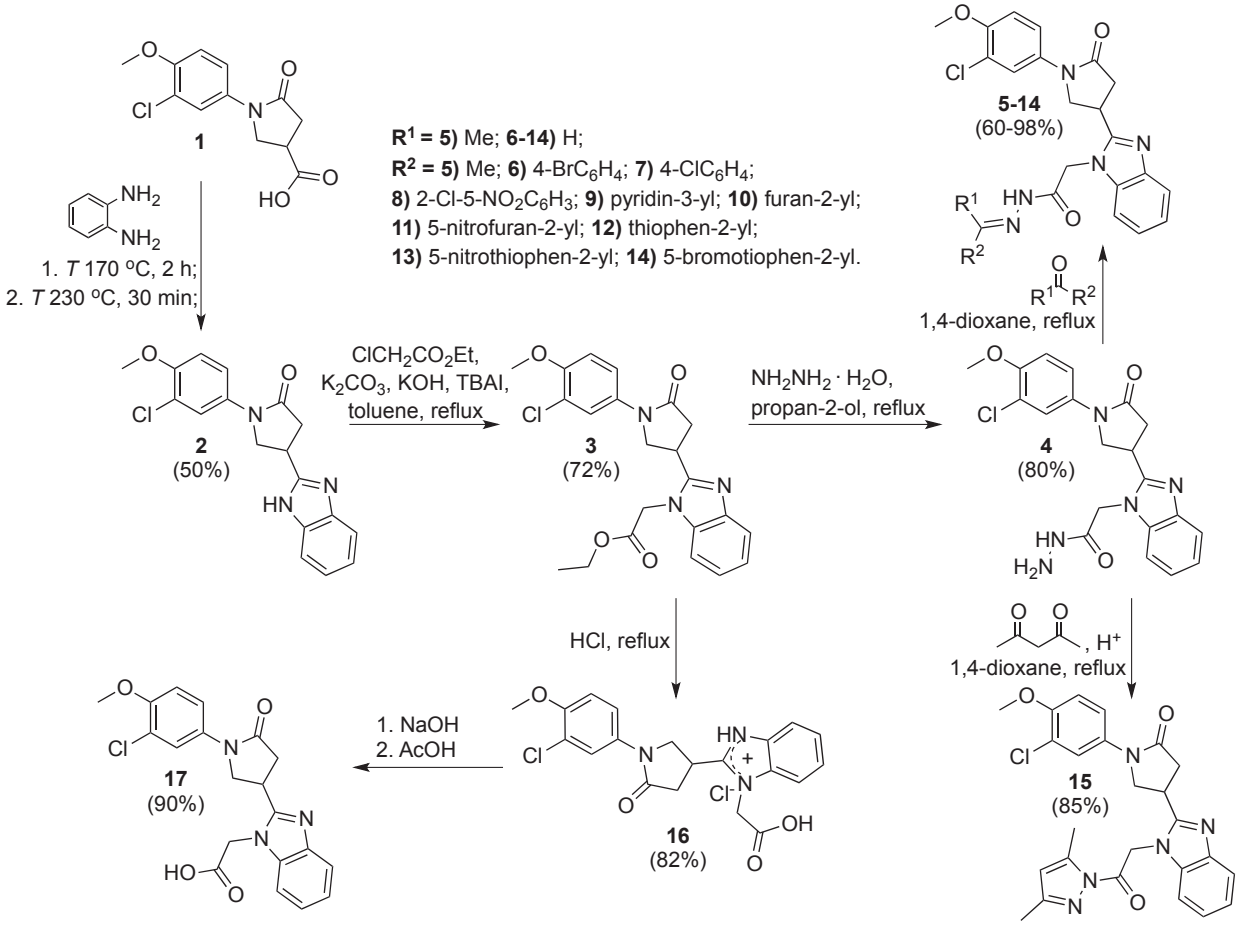
INTRODUCTION

Benzimidazoles are among the nitrogen-containing heterocyclic systems that have great interest in medical chemistry, pharmacology, agrochemical products such as omeprazole, thiabendazole, flubendazole, carbedazole, benomyl, astemidazole, etc. Benzimidazoles are associated with antimicrobial,[1] antifungal,[2] antiviral,[3] anthelmintic,[4] antihypertensive,[5] analgesic,[6] antitumor[7] and other actions. Benzimidazoles are also used in coordination chemistry. [8] The aim of this work was continuation of the synthesis of new bioactive benzimidazole derivatives containing 1-(3-chloro-4-methoxyphenyl)-5-oxo-3-pyrrolidinyl, carboxyalkyl, hydrazone, pyrrole, pyrazole fragments. The most important method for synthesis of a wide range of benzimidazoles is the

condensation of *o*-diaminobenzenes with carboxylic acids or their derivatives.[9] Looking to all the above facts, our studies have been focused toward the synthesis and bio-evaluation of novel benzimidazole, pyrrolidine, and amino acids as possible bioactive molecules.

RESULTS AND DISCUSSION

Benzimidazole derivatives are an important class of compounds that has attracted increasing attention as a source of new antibacterial agents. Modification at the 1- and 2-positions of the benzimidazole moiety has an important effect on biological activity. One of the methods for the synthesis of benzimidazoles is condensation of carboxylic acids with 1,2-diaminobenzenes. In the present work, target compound 1-substituted 3-carboxy-5-oxopyrrolidine **1** was prepared according to the known method by refluxing the corresponding aromatic amine with itaconic acid in water. The compound – 2-substituted benzimidazole **2** – was synthesized by heating a mixture of 4-carboxy-2-pyrrolidinone **1** and 1,2-diaminobenzene at 170–230 °C (Scheme 1), and the obtained product was recrystallized from 1,4-dioxane.



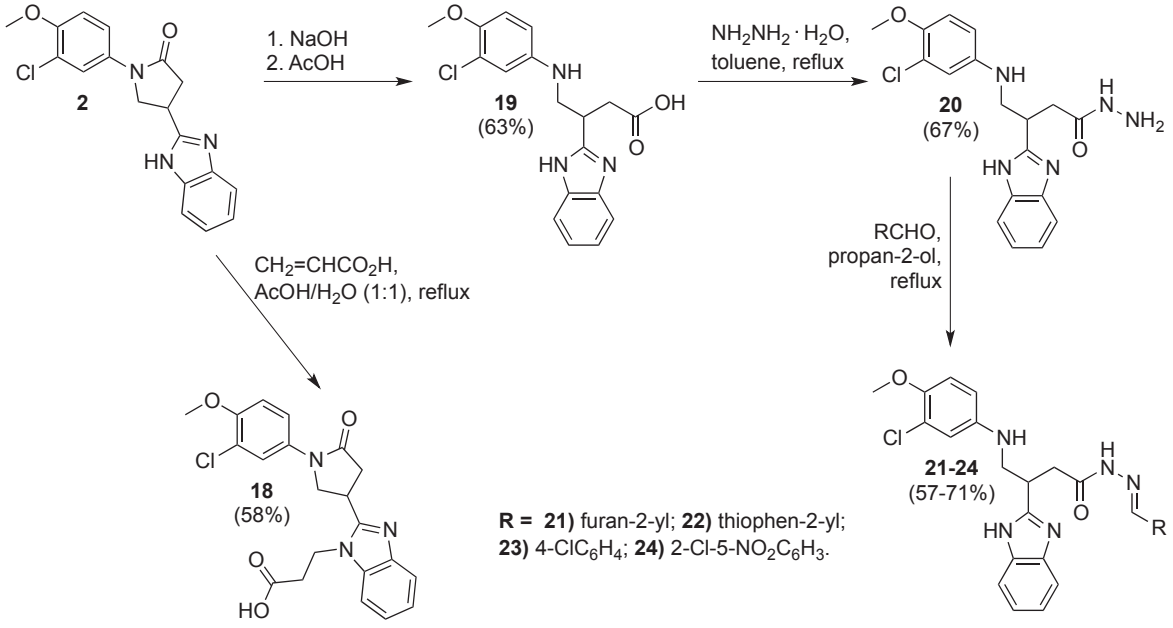
Scheme 1

1,2-Disubstituted benzimidazole **3** was obtained by alkylation of 1-(3-chloro-4-methoxyphenyl)-3-(1*H*-benzimidazol-2-yl)-5-oxopyrrolidine (**2**) with ethyl chloroacetate in toluene in the presence of potassium carbonate, potassium hydroxide and a catalytic amount of

tetrabutylammonium iodide. Series of new hydrazones **5–14** containing benzimidazole and pyrrolidinone moieties were synthesized from 2-{2-[1-(3-chloro-4-methoxyphenyl)-5-oxo-3-pyrrolidinyl]-1*H*-benzimidazol-1-yl}acetohydrazide (**4**) and aromatic, heterocyclic aldehydes or acetone which had been obtained by reaction of the ethyl ester **3** with hydrazine hydrate in refluxing propanol. Analysis of ^{1}H NMR spectra of 1-aryl-3-arylidenehydrazinocarbonyl-5-oxopyrrolidines **5–14** has shown that in DMSO-d_6 solutions there exists a mixture of *E/Z* geometric isomer where the *Z* isomer predominates due to its hindered rotation around the CO-NH bond. [10]

During the reaction of carbohydrazide **4** with 2,4-pentanedione, performed in refluxing 1,4-dioxane in the presence of a catalytic amount of the hydrochloric acid, the *N*-substituted pyrrazole derivative **15** was synthesized. The structure of this compound authenticates spectral data. For example, the formation of a 3,5-dimethylpyrazole ring included into the **15** composition is displayed by the resonance of CH at 110.3 ppm, =C at 132.9 ppm, and 2CH_3 at 13.6 and 13.8 ppm in ^{13}C NMR spectra and singlets at 2.29 and 2.45 ppm (2CH_3), 6.32 ppm (=CH) in ^{1}H NMR spectra. The hydrolysis of the synthesized ester **3** was carried out in refluxing concentrated hydrochloric acid. In these conditions, not only the hydrolysis of the ester group takes place, but also the corresponding benzimidazolium chloride **16** is formed. The latter was converted to the respective base **17** by heating quaternary salt in a sodium hydroxide solution and then acidifying with acetic acid. The IR, ^{1}H, and ^{13}C NMR and mass spectra were in agreement with the suggested structures of compounds **3, 16, 17**.

In the next part of synthesis, carboxyalkylsubstituted benzimidazoles **18, 19** and their derivatives **20–24** were obtained (Scheme 2).



Scheme 2

It was determined that the reaction of 2-substituted benzimidazole derivative **2** with acrylic acid was the most facile in refluxing a 50% acetic acid water solution. The reaction product **18** was separated from the reaction mixture by diluting it with water. It is known that the 5-oxopyrrolidine cycle is not resistant to alkaline hydrolysis.[11] In the present work, the sodium salt of 3-(1*H*-benzimidazol-2-yl)-4-(3-chloro-4-methoxyphenylamino)butanoic acid was formed by the decomposition of the pyrrolidinone cycle of 1-(3-chloro-4-methoxyphenyl)-3-(1*H*-benzimidazol-2-yl)-5-oxopyrrolidine (**2**) in refluxing a 20% water solution of sodium hydroxide. The acidification of the solution with acetic acid up to pH 6 gave a stable 3-(1*H*-benzimidazol-2-yl)-4-(3-chloro-4-methoxyphenylamino)butanoic acid (**19).**

The structural changes of compound **19** have been revealed by a comparison of its ^{13}C NMR spectra with the compound **2** containing a pyrrolidinone ring. The resonance at 173.4 ppm clearly shows the presence of an open-chain compound. Chemical shifts of atoms C-2 and C-3 (CH and CH_2CO) of these compounds are quite close – the difference is only 0.6 ppm, while in a cyclic compound it reaches up to 6.8 ppm. In ^{1}H NMR spectra, the amino acid **19** broad singlet of NH at 5.70 ppm confirmed the existence of an open chain. Finally, hydrazones **21–24** of γ-amino acid were obtained from carbohydrazide **20** which was obtained by boiling with hydrazine hydrate in toluene. Analysis of ^{1}H NMR spectra of hydrazones **21-24** has shown that in DMSO-d_6 solutions there exists a mixture of *E/Z* rotamers where the *Z* isomer predominates due to its hindered rotation around the CO-NH bond.

Antibacterial activity of the synthesized compounds

Emerging infectious diseases and the increasing number of multi-drug-resistant microbial pathogens still make the treatment of infectious diseases an important and challenging problem.[12] In spite of the large number of antibiotics and chemotherapeutics available for medical use, the emergence of old and new antibiotic-resistant bacterial strains in the last decades confirms a substantial need for new classes of antibacterial agents.[13] Most of the antibiotics currently in use can be classified as follows: β-lactam and glycopeptide antibiotics targeting cell wall biosynthesis; aminoglycoside, tetracycline, and macrolide antibiotics targeting protein synthesis; and fluoroquinolones targeting DNA gyrase and topoisomerase. To tackle the problem of drug resistance, one can focus on these proven targets and develop new drugs to overcome drug-induced resistance caused by the mutations of the targets or the modification of the antibiotics.[14]

The minimal inhibition concentration (MIC, μg/mL) values are shown in Table 1.

Table 1. Antibacterial activity *in vitro* (MIC μg/mL) of the synthesized compounds

Compound	*Bacillus cereus*	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
2	+	31.25	7.8	0.485
3	+	+	+	7.8
4	+	7.8	125	125
5	+	62.5	15.6	125
6	+	0.12	+	+
7	+	+	+	7.8
8	+	0.485	+	+
9	+	+	+	+
10	+	0.24	+	+
11	+	0.485	+	1.95
12	+	+	+	+
13	+	+	+	+
14	+	+	+	+
15	+	+	+	+
17	500	62.5	125	125
18	500	125	125	62.5
19	500	+	125	125
20	500	125	125	125
21	500	62.5	+	125
22	500	+	125	500
23	+	+	+	+
24	+	+	+	+
Oxytetracycline	62.5	62.5	62.5	62.5

+ – growth of microorganisms

It is noteworthy to mention here that the entire set of test compounds showed a marked to considerable inhibition pattern against the bacterial strains and some cases showed a more potent activity than the reference standard. The data show (Table 1) that a number of the investigated compounds has antibacterial properties. It has been found that only compounds **17–22** containing benzimidazole ring carboxy alkyl alternatives or γ--amino acid derivatives have a weak antibacterial activity against *B. cereus*

bacteria (its cell wall consists of a neutral polysaccharide composed of *N*-acetylglucosamine, *N*-acetylmannosamine) at the 500 μg/mL concentration. A similar tendency was observed in *S. aureus* (its cell wall consists of a thick peptidoglycan layer) and *P. aeruginosa* (has an outer membrane which contains Protein F) bacteria. The MIC value of the compounds of 125 μg/mL was obtained.

From Table 1, it was inferred that compounds **17-22** showed a more potent activity against *P. aeruginosa*, equi-potent to *S. aureus*, *E. coli* and a considerable to lesser activity against *B. cereus*.

The most active against *E. coli* bacteria (its cell wall that consists of an outer membrane containing lipopolysaccharides) were hydrazide **4** and hydrazones **5, 6, 8, 10, 11**. Hydrazide **4** possessed more potent antibacterial activity against *E. coli, S. aureus, P. aeruginosa*, while hydrazones **5**, **6**, **8**, **10** exhibited good activity only against *E. coli*. It was observed that antibacterial activity also was increased by hydrazones containing 2-chloro-5-nitrophenyl, furan-2-yl and 5-nitro-furan-2-yl substituents. These active compounds within their structure have a pyrrolidinone cycle, and its disruption by γ-amino acid reduce the antibacterial effect.

The inhibition of *E. coli* bacteria was excellent when the MIC was 0.12 μg/mL of hydrazone **6** with 4-bromophenyl substituent. The minimal bactericidal concentration (MBC, μg/mL) values are shown in Table 2.

Table 2. Antibacterial activity *in vitro* of the synthesized (MBC, μg/mL) compounds

Compound	*Bacillus cereus*	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
2	125	62.5	7.8	1.95
3	+	31.25	31.25	7.8
4	+	7.80	250	125
5	+	62.5	15.6	250
6	+	0.24	15.6	31.25
7	+	31.25	250	15.6
8	+	0.485	500	62.5
9	+	31.25	500	62.5
10	+	0.24	250	125
11	+	0.485	250	1.95
12	+	125	3.90	31.25
13	+	125	7.80	15.6
14	+	125	62.5	125

15	+	3.9	250	15.6
17	500	125	125	125
18	500	125	125	125
19	500	15.6	125	125
20	500	125	125	125
21	500	62.5	125	125
22	500	62.5	125	125
23	+	250	62.5	15.6
24	+	250	62.5	7.8
Oxytetracycline	62.5	62.5	62.5	62.5

+ – growth of microorganisms

B. cereus was sensitive to compounds **2, 17-22**. The most active compounds were hydrazide **4** and hydrazones **6**, **8**, **10, 11** and **15** against *E. coli* and **2**, **3**, **7**, **10**, **11**, **12** and **15** derivatives, their structure having a pyrrolidinone cycle against *P. aeruginosa*. It was found that γ-amino acid derivatives **19**–**24** showed a moderate effect against the bacteria *E. coli, S. aureus,* and *P. aeruginosa*. The antibiotic was effective at 62.5 μg/mL concentration of MIC and MBC against the test strains *S. aureus*, *B. cereus, E. coli* and *P. aeruginosa.*

The results showed that about 50% of the investigated compounds as bactericides were more active than oxytetracycline. For example, compound **6**, as compared with oxytetracycline, exhibited active antibacterial activity against *E. coli* when the MBC = 0.24 μg/mL, against *S. aureus* – MBC = 15.6 μg/mL, and against *P. aeruginosa* – MBC = 31.25 μg/mL. Therefore, we can conclude that the synthesized compounds are characterized by a strong bactericidal effect, even at low concentrations.

CONCLUSIONS

A series of new 2- and 1,2-disubstituted benzimidazoles have been synthesized by transformation of 1-(3-chloro-4-methoxyphenyl)-3-(1*H*-benzimidazol-2-yl)-5-oxopyrrolidine. The synthesized compounds are characterized by a strong bactericidal effect, even at low concentrations, and this is a pre-requisite for the further synthesis and investigation of substituted benzimidazole derivatives of similar structure. Benzimidazole derivatives with pyrrolidinone moiety are characterized by higher antibacterial activity in comparison with the non-cyclic compounds – *N*-substituted *γ*-amino acid derivatives except 2-{2-[1-(3-chloro-4-methoxyphenyl)-5-oxo-3-pyrrolidinyl]-1*H*-benzimidazol-1-yl}acetic acid and 3-{2-[1-(3-chloro-4-methoxyphenyl)-5-oxo-3-pyrrolidinyl]-1*H*-benzimidazol-1-yl}propanoic acid, which

contain benzimidazole ring and have the carboxy alkyl group at the 1-position. Though γ-amino acid derivatives showed moderate effect, these compounds were effective against almost all screened bacteria.

EXPERIMENTAL

Microbiology

The antibacterial activity of the compounds **2–15** and **17–24** was determined by testing different concentrations against gram-positive spore-forming rods *Bacillus cereus* (ATCC 11778), gram-positive cocci *Staphylococcus aureus* (ATCC 9144), gram-negative rods *Escherichia coli* (ATCC 8739) and *Pseudomonas aeruginosa* (NCTC 6750) by the broth and spread-plate methods. The test bacteria (*B. cereus*, *S. aureus*, *E. coli*, and *P. aeruginosa*) were streaked out on TSA plates and incubated at 37 °C for 24 h. A representative colony was placed in 5 mL of TBS and incubated at 37°C for 24 h. *S. aureus, B. cereus, E. coli* and *P. aeruginosa* cultures containing 10^8 CFU/mL (colony-forming units corresponding to McFarland's) were prepared by dilution with TSB and used for antimicrobial tests. Solutions of the test compounds were prepared in dimethyl sulfoxide at 500, 250, 125, 62.5, 31.25, 15.6, 7.8, 3.9, 1.95, 0.97, 0.485, 0.24 and 0.12 μg/mL. The test organisms (100 μL) were added to each tube and incubated at 37 °C for 24 h. At the end of this period, a small amount of the diluted mixture from each tube was pulled out and spread on TSA. The plates were incubated at 37 °C for 48 h. The growth of bacterial cells was noted on agar plates. The lowest concentration of the bactericidal material at which no growth was observed was determined as the minimum bactericidal concentration (MBC) value. Oxytetracycline was used as a control in antibacterial activity tests of the synthesized compounds.

Synthesis

General Methods. The melting points were determined on a MEL-TEMP (Electrothermal, Bibby Scientific Company, Burlington, NJ, USA) melting point apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 on a Brucker Avance III (400 MHz, 101 MHz) and Brucker Avance III (700 MHz, 176 MHz) spectrometers. Chemical shifts (δ) are reported in parts per million (ppm) calibrated from TMS (0 ppm) as an internal standard for ^{1}H NMR, and DMSO-d_6 (39.43 ppm) for ^{13}C NMR. Mass spectra were obtained either on a Waters (Micromass, Milford, MA, USA) ZQ 2000 Spectrometer using ESI (20 eV) ionization or on a Bruker maXis UHR-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) with ESI negative ionization. Samples were introduced using a Waters Acquity UPLC system. Methanol was used as an eluent for sample introduction. The capillary voltage was maintained at +4000 V with the end plate offset at −500 V. Nitrogen was used as the drying and nebulizing gases at a flow rate of 10.0 L/min and a pressure of 2.0 bar, respectively. IR spectra (ν, cm^{-1}) were recorded on a Perkin–Elmer Spectrum BX FT–IR spectrometer using KBr tablets. Elemental

analyses (C, H, N) were performed on an Elemental Analyzer CE-440 (Exeter Analytical, Inc., North Chelmsford, MA, USA). The reaction course and the purity of the synthesized compounds was monitored by TLC using aluminium plates pre-coated with silica gel 60 F_{254} (MerckKGaA, Darmstadt, Germany). Synthesis-starting reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Fluka (Buchs, Switzerland).

4-(1*H*-Benzimidazol-2-yl)-1-(3-chloro-4-methoxylphenyl)pyrrolidin-2-one (2). A mixture of 1-(3-chloro-4-methoxyphenyl)-5-oxopyrrolidine-3-carboxylic acid (**1**) (62.03 g, 0.23 mol) and *o*-phenylenediamine (32.44 g, 0.30 mol) was heated at 170 °C for 2 h and then at 230 °C for 30 min. The formed hard and dry residue was poured with 5% (300 mL) aqueous potassium carbonate solution and refluxed for 10 min. The formed product was filtered off, washed with water, and recrystallized from 1,4-dioxane to afford white solid, yield 39.00 g (50%); mp 249–251 °C; IR (KBr): ν_{max} 3173 (NH), 1690 (CO), 1606 (CN) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 2.92–3.08 (m, 2H, CH_2CO), 3.84 (s, 3H, OCH_3), 3.94–4.06 (m, 1H, CHCN), 4.15–4.30 (m, 2H, N*C*H_2CH), 7.12–7.89 (m, 7H, aromatic H), 12.45 (br. s, 1H, NH); ^{13}C NMR (101 MHz, DMSO-*d*$_6$): δ 30.6 (CH), 37.4 (*C*H_2CO), 52.3 (CH_2N), 56.2 (OCH_3), 112.7, 119.4, 120.7, 121.4, 121.6, 132.9, 151.1, 155.0 (aromatic C), 171.9 (CO); *Anal.* Calcd for $C_{18}H_{16}ClN_3O_2$: C, 63.25; H, 4.72; N, 12.29%. Found: C, 63.56; H, 4.70; N, 12.20%.

Ethyl 2-{2-[1-(3-chloro-4-methoxylphenyl)-5-oxo-3-pyrrolidinyl]-1*H*-benzimidazol-1-yl}acetate (3). A mixture of compound **2** (10.25 g, 30 mmol), potassium carbonate (8.29 g, 60 mmol), potassium hydroxide powder (0.84 g, 15 mmol), tetrabutylammonium iodide (0.30 g) and toluene (100 mL) was heated to boiling, then in 10 min by stirring, chloroacetic acid ethyl ester (4.29 g, 3.75 mL, 35 mmol) was added. The mixture was refluxed for 5 h. A hot mixture was filtered off, and the filtrate was cooled. After cooling, the precipitated compound **3** was filtered off, washed with toluene, and recrystallized from toluene to afford white solid, yield 9.25 g (72%); mp 182–184 °C; IR (KBr): ν_{max} 1730, 1703 (CO), 1607 (CN) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 1.23 (t, *J* = 7.1 Hz, 3H, CH_2*C*H_3), 2.86–3.05 (m, 2H, $COCH_2$), 3.84 (s, 3H, OCH_3), 4.04–4.28 (m, 5H, CHCN, *C*H_2CH_3, N*C*H_2CH), 5.30 (s, 2H, NCH_2CO), 7.15–7.90 (m, 7H, aromatic H); ^{13}C NMR (101 MHz, DMSO-*d*$_6$): δ 14.1 (CH_2*C*H_3), 28.5 (CH), 37.4 (*C*H_2CO), 44.4 (N*C*H_2CO), 52.1 (CH_2N), 56.2 (OCH_3), 61.5 (*C*H_2CH_3), 110.1, 112.7, 118.9, 119.4, 120.7, 121.4, 121.9, 122.4, 132.8, 135.8, 141.7, 151.2, 155.4 (aromatic C), 168.4, 171.7 (CO); HRMS (ESI): *m/z* calcd for $C_{22}H_{22}ClN_3O_4$+H^+: 428.1372 [M+H^+]; found: 428.1370; *Anal.* Calcd for $C_{22}H_{22}ClN_3O_4$: C, 61.75; H, 5.18; N, 9.82%. Found: C, 62.00; H, 5.30; N, 9.96%.

2-{2-[1-(3-Chloro-4-methoxyphenyl)-5-oxopyrrolidin-3-yl]-1*H*-benzimidazol-1-yl}acetohydrazide (4). A mixture of ester **3** (8.56 g, 20 mmol), hydrazine monohydrate (2.50 g, 2.46 mL, 50 mmol) was

refluxed in propan-2-ol (180 mL) for 20 h. The reaction mixture was cooled, the precipitated was filtered off, washed with propan-2-ol, and recrystallized from propan-2-ol–water mixture (2:1) to afford white solid, yield 6.64 g (80%); mp 251–254 °C; IR (KBr): ν_{max} 3307, 3108 (NH, NH_2), 1687, 1664 (CO), 1606 (CN) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.94–3.07 (m, 2H, $COCH_2$), 3.84 (s, 3H, OCH_3), 4.08–4.29 (m, 3H, CHCN, NCH_2CH), 4.41 (s, 2H, NCH_2CO), 4.92 (s, 2H, NH_2), 7.14–7.90 (m, 7H, aromatic H), 9.61 (s, 1H, NH); ^{13}C NMR (101 MHz, DMSO-d_6): δ 28.6 (CH), 37.5 (CH_2CO), 44.4 (NCH_2CO), 52.3 (CH_2N), 56.2 (OCH_3), 110.0, 112.7, 118.8, 119.4, 120.7, 121.4, 121.8, 122.2, 132.9, 135.8, 141.8, 151.1, 155.8 (aromatic C), 166.0, 171.9 (CO); *Anal.* Calcd for $C_{20}H_{20}ClN_5O_3$: C, 58.04; H, 4.87; N, 16.92%. Found: C, 58.23; H, 4.99; N, 16.78%.

2-{2-[1-(3-Chloro-4-methoxyphenyl)-5-oxo-3-pyrrolidinyl]-1*H*-benzimidazol-1-yl}-*N′*-(1-methylethylidene)acetohydrazide (5). A mixture of acetohydrazide **4** (1.04 g, 2.5 mmol) and acetone (36.71 mL, 0.5 mol) was heated under reflux for 5 h. After cooling the precipitate was isolated by filtration, washed with acetone, and recrystallized from 1,4-dioxane to afford white solid, yield 0.88 g (78%); mp 173–174 °C; IR (KBr): ν_{max} 3024 (NH), 1696, 1660 (CO), 1807, 1574 (CN) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): (*Z/E* isomeric mixture, 65/35): δ 1.92, 1.95, 1.96, 2.02 (4s, 6H, $NC(CH_3)_2$), 2.92–3.03 (m, 2H, $COCH_2$), 3.84 (s, 3H, OCH_3), 3.99–4.29 (m, 3H, CHCN, NCH_2CH), 5.13 (s, 0.7H, NCH_2CO), 5.41 (s, 1.3H, NCH_2CO), 7.10–7.91 (m, 7H, aromatic H), 10.60 (s, 0.35H, NH), 10.69 (s, 0.65H, NH); ^{13}C NMR (101 MHz, DMSO-d_6): δ 17.3, 17.8, 25.0, 25.3 ($C(CH_3)_2$), 28.5 (CH), 37.5 (CH_2CO), 44.5 (NCH_2CO), 52.3 (CH_2N), 56.3 (OCH_3), 109.9, 110.0, 112.7, 118.7, 119.4, 120.7, 121.4, 121.6, 122.1, 132.9, 135.9, 136.2, 141.8, 151.1, 152.2, 156.0 (aromatic C), 168.5, 171.9 (CO); *Anal.* Calcd for $C_{23}H_{24}ClN_5O_3$: C, 60.86; H, 5.33; N, 15.43%. Found: C, 60.36; H, 5.47; N, 15.10%.

General procedure for the preparation of hydrazones 6–14.

A mixture of hydrazide **4** (1.04 g, 2.5 mmol) and the appropriate aldehyde (3 mmol) in 1,4-dioxane (20 mL) was heated under reflux for 6 h and cooled down. The crystals formed were filtered off and washed with 1,4-dioxane.

***N′*-[(4-Bromophenyl)methylidene]-2-{2-[1-(3-chloro-4-methoxyphenyl)-5-oxo-3-pyrrolidinyl]-1*H*-benzimidazol-1-yl}acetohydrazide (6)** was prepared from **4** and 4-bromobenzaldehyde. Recrystallized from 1,4-dioxane to afford white solid, yield 1.26 g (87%); mp 189–191 °C; IR (KBr): ν_{max} 3091 (NH), 1690, 1658 (CO), 1610, 1591 (CN) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6), (*Z/E* isomeric mixture, 75/25): δ 2.90–3.05 (m, 2H, $COCH_2$), 3.84 (s, 3H, OCH_3), 4.06–4.28 (m, 3H, CHCN, NCH_2CH), 5.13 (s, 0.5H, NCH_2CO), 5.60 (s, 1.5H, NCH_2CO), 7.12–7.88 (m, 11H, aromatic H), 8.07 (s, 0.75H, N=CH), 8.26 (s, 0.25H, N=CH), 11.94 (s, 0.75H, NH), 12.05 (s, 0.25H, NH); ^{13}C NMR (101 MHz, DMSO-d_6): δ 28.5 (CH), 37.5 (CH_2CO), 44.2 (NCH_2CO), 52.3 (CH_2N), 56.2 (OCH_3), 112.7, 119.4, 120.7, 121.4, 121.7,

129.1, 131.8, 132.9, 133.3, 141.9, 143.2, 151.1, 156.0 (aromatic C), 168.6, 171.8 (CO); *Anal.* Calcd for $C_{27}H_{23}BrClN_5O_3$: C, 55.83; H, 3.99; N, 12.06%. Found: C, 55.54; H, 4.06; N, 11.90%.

***N′*-[(4-Chlorophenyl)methylidene]-2-{2-[1-(3-chloro-4-methoxyphenyl)-5-oxo-3-pyrrolidinyl]-1*H*-benzimidazol-1-yl}acetohydrazide (7)** was prepared from **4** and 4-chlorobenzaldehyde. Recrystallized from propan-2-ol to afford white solid, yield 1.13 g (84%); mp 275–277 °C; IR (KBr): ν_{max} 3094 (NH), 1691, 1656 (CO), 1614, 1572 (CN) cm^{-1}; ^1H NMR (700 MHz, DMSO-d_6), (*Z/E* isomeric mixture, 75/25): δ 2.89–3.05 (m, 2H, $COCH_2$), 3.84 (s, 3H, OCH_3), 4.07–4.29 (m, 3H, CHCN, NCH_2CH), 5.13 (s, 0.5H, NCH_2CO), 5.60 (s, 1.5H, NCH_2CO), 7.12–7.90 (m, 11H, aromatic H), 8.09 (s, 0.75H, N=CH), 8.28 (s, 0.25H, N=CH), 11.91 (s, 0.75H, NH), 12.02 (s, 0.25H, NH); ^{13}C NMR (176 MHz, DMSO-d_6): δ 28.5 (CH), 37.5 (CH_2CO), 44.2 (NCH_2CO), 52.3 (CH_2N), 56.2 (OCH_3), 110.1, 112.7, 118.7, 119.4, 120.7, 121.4, 121.6, 122.1, 128.8, 128.9, 132.9, 133.0, 134.5, 136.2, 141.8, 143.1, 151.1, 155.9 (aromatic C), 168.6, 171.8 (CO); *Anal.* Calcd for $C_{27}H_{23}Cl_2N_5O_3$: C, 60.46; H, 4.32; N, 13.06%. Found: C, 60.35; H, 4.15; N, 13.25%.

***N′*-[(2-Chloro-5-nitrophenyl)methylidene]-2-{2-[1-(3-chloro-4-methoxyphenyl)-5-oxo-3-pyrrolidiny]-1*H*-benzimidazol-1-yl}acetohydrazide (8)** was prepared from **4** and 2-chloro-5-nitrobenzaldehyde. Recrystallized from 1,4-dioxane to afford white solid, yield 0.94 g (65%); mp 302–303 °C; IR (KBr): ν_{max} 3092 (NH), 1697, 1651 (CO), 1607, 1599 (CN) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6), (*Z/E* isomeric mixture, 70/30): δ 2.87–3.07 (m, 2H, $COCH_2$), 3.84 (s, 3H, OCH_3) 4.07–4.34 (m, 3H, CHCN, NCH_2CH), 5.19 (s, 0.6H, NCH_2CO), 5.57 (s, 1.4H, NCH_2CO), 7.10–8.89 (m, 11H, aromatic H and N=CH), 12.22 (s, 0.7H, NH), 12.43 (s, 0.3H, NH); *Anal.* Calcd for $C_{27}H_{22}Cl_2N_6O_5$: C, 55.78; H, 3.81; N, 14.45%. Found: C, 55.96; H, 3.58; N, 14.11%.

2-{2-[1-(3-Chloro-4-methoxyphenyl)-5-oxo-3-pyrrolidinyl]-1*H*-benzimidazol-1-yl}-*N′*-(3-pyridinylmethylidene)acetohydrazide (9) was prepared from **4** and pyridine-3-carbaldehyde. Recrystallized from 1,4-dioxane to afford white solid, yield 0.75 g (60%); mp 264–265 °C; IR (KBr): ν_{max} 3090 (NH), 1691, 1661 (CO), 1615, 1590, 1569 (CN) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6), (*Z/E* isomeric mixture, 70/30): δ 2.90–3.05 (m, 2H, $COCH_2$), 3.84 (s, 3H, OCH_3), 4.07–4.30 (m, 3H, CHCN, NCH_2CH), 5.15 (s, 0.6H, NCH_2CO), 5.64 (s, 1.4H, NCH_2CO), 7.13–8.66 (m, 11H, aromatic H), 8.86 (s, 0.3H, N=CH), 8.99 (s, 0.7H, N=CH), 12.04 (s, 0.7H, NH), 12.15 (s, 0.3H, NH); ^{13}C NMR (101 MHz, DMSO-d_6): δ 28.5 (CH), 37.5 (CH_2CO), 44.3 (NCH_2CO), 52.3 (CH_2N), 56.2 (OCH_3), 110.2, 112.7, 119.4, 120.7, 121.4, 121.7, 123.9, 130.0, 132.9, 133.8, 136.2, 141.6, 141.9, 148.7, 150.7, 151.1, 156.0 (aromatic C), 168.8, 171.8 (CO); HRMS (ESI): *m/z* calcd for $C_{26}H_{23}ClN_6O_3+H^+$: 503.1593 [M+H^+]; found: 503.1588; *Anal.* Calcd for $C_{26}H_{23}ClN_6O_3$: C, 62.09; H, 4.61; N, 16.71%. Found: C, 62.32; H, 4.53; N, 16.77%.

2-{2-[1-(3-Chloro-4-methoxyphenyl)-5-oxo-3-pyrrolidinyl]-1*H*-benzimidazol-1-yl}-*N′*-(2-furylmeth-

ylidene)acetohydrazide (10) was prepared from **4** and 2-furaldehyde. Recrystallized from 1,4-dioxane to afford white solid, yield 1.21 g (98%); mp 268–269 °C; IR (KBr): ν_{max} 3062 (NH), 1690, 1662 (CO), 1616, 1610 (CN) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6), (*Z/E* isomeric mixture, 70/30): δ 2.90–3.08 (m, 2H, $COCH_2$), 3.84 (s, 3H, OCH_3), 4.06–4.29 (m, 3H, CHCN, NCH_2CH), 5.12 (s, 0.6H, NCH_2CO), 5.49 (s, 1.4H, NCH_2CO), 6.60–7.91 (m, 10H, aromatic H), 7.98 (s, 0.7H, N=CH), 8.17 (s, 0.3H, N=CH), 11.82 (s, 0.7H, NH), 11.92 (s, 0.3H, NH); ^{13}C NMR (101 MHz, DMSO-d_6): δ 28.5 (CH), 37.5 (CH_2CO), 44.2 (NCH_2CO), 52.3 (CH_2N), 56.2 (OCH_3), 110.1, 112.3, 112.7, 114.3, 118.7, 119.4, 120.7, 121.4, 121.7, 122.2, 132.9, 134.6, 136.2, 141.8, 145.3, 148.9, 151.1, 156.0 (aromatic C), 168.2, 171.9 (CO); *Anal.* Calcd for $C_{25}H_{22}ClN_5O_4$: C, 61.04; H, 4.51; N, 14.24%. Found: C, 60.89; H, 4.26; N, 14.07%.

2-{2-[1-(3-Chloro-4-methoxyphenyl)-5-oxo-3-pyrrolidinyl]-1*H*-benzimidazol-1-yl}-*N′*-[(5-nitro-2-furyl)methylidene]acetohydrazide (11) was prepared from **4** and 5-nitro-2-furaldehyde. Recrystallized from 1,4-dioxane to afford white solid, yield 1.13 g (84%); mp 291–292 °C; IR (KBr): ν_{max} 3111 (NH), 1653, 1699, 173 (CO), 1606, 1568 (CN) cm^{-1}; ^1H NMR (700 MHz, DMSO-d_6): (*Z/E* isomeric mixture, 70/30): δ 2.89–3.06 (m, 2H, $COCH_2$), 3.84 (s, 3H, OCH_3), 4.06–4.30 (m, 3H, CHCN, NCH_2CH), 5.18 (s, 0.6H, NCH_2CO), 5.56 (s, 1.4H, NCH_2CO), 7.07–7.92 (m, 9H, aromatic H), 8.05 (s, 0.7H, N=CH), 8.26 (s, 0.3H, N=CH), 12.23 (s, 0.7H, NH), 12.31 (s, 0.3H, NH); *Anal.* Calcd for $C_{25}H_{21}ClN_6O_6$: C, 55.92; H, 3.94; N, 15.65%. Found: C, 55.81; H, 3.76; N, 15.43%.

2-{2-[1-(3-Chloro-4-methoxyphenyl)-5-oxo-3-pyrrolidinyl]-1*H*-benzimidazol-1-yl}-*N′*-[2-thienyl-methylidene]acetohydrazide (12) was prepared from **4** and 2-thiophenecarbaldehyde. Recrystallized from 1,4-dioxane to afford white solid, yield 1.05 g (83%); mp 287–288 °C; IR (KBr): ν_{max} 3087 (NH), 1688, 1661 (CO), 1617, 1570 (CN) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6), (*Z/E* isomeric mixture, 70/30): δ 2.93–3.07 (m, 2H, $COCH_2$), 3.84 (s, 3H, OCH_3), 4.06–4.31 (m, 3H, CHCN, NCH_2CH), 5.11 (s, 0.6H, NCH_2CO), 5.49 (s, 1.4H, NCH_2CO), 7.11–7.91 (m, 10H, aromatic H), 8.28 (s, 0.7H, N=CH), 8.49 (s, 0.3H, N=CH), 11.85 (s, 0.7H, NH), 11.92 (s, 0.3H, NH); ^{13}C NMR (101 MHz, DMSO-d_6): δ 28.5 (CH), 37.5 (CH_2CO), 43.9 (NCH_2CO), 52.3 (CH_2N), 56.2 (OCH_3), 110.6, 112.7, 118.7, 119.4, 120.7, 121.4, 122.2, 128.0, 128.9, 131.0, 132.9, 136.1, 138.5, 139.7, 141.8, 151.1, 156.0 (aromatic C), 168.1, 171.8 (CO); *Anal.* Calcd for $C_{25}H_{22}ClN_5O_3S$: C, 59.11; H, 4.37; N, 13.79%. Found: C, 59.26; H, 4.52; N, 13.88%.

2-{2-[1-(3-Chloro-4-methoxyphenyl)-5-oxo-3-pyrrolidinyl]-1*H*-benzimidazol-1-yl}-*N′*-[(5-nitro-2-thienyl)methylidene]acetohydrazide (13) was prepared from **4** and 5-nitro-2-thiophenecarbaldehyde. Recrystallized from 1,4-dioxane to afford white solid, yield 1.13 g (82%); mp 288–289 °C; IR (KBr): ν_{max} 3107 (NH), 1692, 1651 (CO), 1617, 1587 (CN) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6), (*Z/E* isomeric mixture, 70/30): δ 2.87–3.08 (m, 2H, $COCH_2$), 3.84 (s, 3H, OCH_3), 4.05–4.30 (m, 3H, CHCN, NCH_2CH),

5.16 (s, 0.6H, NCH_2CO), 5.57 (s, 1.4H, NCH_2CO), 7.10–8.19 (m, 9H, aromatic H), 8.27 (s, 0.7H, N=CH), 8.53 (s, 0.3H, N=CH), 12.26 (s, 0.7H, NH), 12.34 (s, 0.3H, NH); ^{13}C NMR (101 MHz, DMSO-d_6): δ 28.5 (CH), 37.5 (CH_2CO), 44.1 (NCH_2CO), 52.3 (CH_2N), 56.2 (OCH_3), 112.7, 119.4, 120.7, 121.4, 121.7, 122.2; 129.6, 130.6, 132.9, 136.2, 137.8, 141.9, 146.2, 150.9, 151.1, 156.0 (aromatic C), 168.8, 171.8 (CO); *Anal.* Calcd for $C_{25}H_{21}ClN_6O_5S$: C, 54.30; H, 3.83; N, 15.20%. Found: C, 54.32; H, 3.95; N, 15.08%.

***N′*-[(5-Bromo-2-thienyl)methylidene]-2-{2-[1-(3-chloro-4-methoxyphenyl)-5-oxo-3-pyrrolidinyl]-1*H*-benzimidazol-1-yl}acetohydrazide (14)** was prepared from **4** and 5-bromothiophene-2-carbaldehyde. Recrystallized from 1,4-dioxane to afford white solid, yield 1.25 g (85%); mp 283–284 °C; IR (KBr): ν_{max} 3105 (NH), 1691, 1656 (CO), 1617, 1599 (CN) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6), (*Z*/*E* isomeric mixture, 70/30): δ 2.89–3.07 (m, 2H, $COCH_2$), 3.84 (s, 3H, OCH_3), 4.00–4.30 (m, 3H, CHCN, NCH_2CH), 5.10 (s, 0.6H, NCH_2CO), 5.47 (s, 1.4H, NCH_2CO), 7.13–7.90 (m, 9H, aromatic H), 8.19 (s, 0.7H, N=CH), 8.42 (s, 0.3H, N=CH), 11.92 (s, 0.7H, NH), 11.99 (s, 0.3H, NH); ^{13}C NMR (101 MHz, DMSO-d_6): δ 28.5 (CH), 37.5 (CH_2CO), 43.9 (NCH_2CO), 52.3 (CH_2N), 56.2 (OCH_3), 112.7, 114.7, 118.8, 119.4, 120.7, 121.4, 121.7, 122.2; 131.4, 131.5, 132.9, 136.1, 138.7, 140.5, 141.8, 151.1, 156.0 (aromatic C), 168.8, 171.8 (CO); *Anal.* Calcd for $C_{25}H_{21}BrClN_5O_3S$: C, 51.16; H, 3.61; N, 11.93%. Found: C, 51.38; H, 3.83; N, 11.74%.

1-(3-Chloro-4-methoxyphenyl)-4-{1-[2-(3,5-dimethyl-1*H*-pyrazol-1-yl)-2-oxoethyl]-1*H*-benzimidazol-2-yl}-2-pyrrolidinone (15). A mixture of acetohydrazide **4** (1.04 g, 2.5 mmol), 2.4-pentanedione (1.50 g, 1.54 mL, 15 mmol), 1,4-dioxane (16 mL) and conc. hydrochloric acid (0.1 mL) was heated under reflux for 4 h, then cooled, diluted with water. The precipitate was filtered off, washed with water, recrystallized from 1,4-dioxane to afford white solid, yield 1.01 g (85%); mp 220–221 ºC; IR (KBr): ν_{max} 1721, 1706 (CO), 1605, 1593 (CN) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.29, 2.45 (2s, 6H, $2CCH_3$), 2.87–3.02 (m, 2H, $COCH_2$), 3.84 (s, 3H, OCH_3), 4.09–4.25 (m, 3H, CHCN, NCH_2CH), 5.94 (s, 2H, NCH_2CO), 6.32 (s, 1H, CCHC), 7.13–7.87 (m, 7H, aromatic H); ^{13}C NMR (101 MHz, DMSO-d_6): δ 13.6, 13.8 ($2CH_3$), 28.4 (CH), 37.6 (CH_2CO), 46.3 (NCH_2CO), 52.3 (CH_2N), 56.2 (OCH_3), 110.3, 111.7, 112.7, 118.8, 119.4, 120.7, 121.3, 121.9, 122.3, 132.9, 136.0, 141.8, 144.1, 151.1, 152.8, 155.9 (aromatic C, pyrazole C), 167.6, 171.8 (CO); *Anal.* Calcd for $C_{25}H_{24}ClN_5O_3$: C, 62.83; H, 5.06; N, 14.65%. Found: C, 62.99; H, 5.16; N, 14.32%.

1-(Carboxymethyl)-2-[1-(3-chloro-4-methoxyphenyl)-5-oxo-3-pyrrolidinyl]-1*H*-benzimidazol-1-ium chloride (16). A mixture of compound **3** (1.07 g, 2.5 mmol) and concentrated hydrochloric acid (10 mL) was refluxed for 4.5 h. The reaction mixture was cooled, the residue was filtered and washed with water, recrystallized from propan-2-ol to afford white solid, yield 0.89 g (82%); mp 250–251 °C; IR (KBr): ν_{max}

3380 (OH), 1737, 1699 (CO), 1602 (CN) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.97–3.17 (m, 2H, $COCH_2$), 3.85 (s, 3H, OCH_3), 4.19–4.51 (m, 3H, CHCN, NCH_2CH), 5.48 (s, 2H, CH_2COOH), 7.12–7.90 (m, 7H, aromatic H), 13.79 (br. s, 1H, OH); *Anal.* Calcd for $C_{20}H_{19}Cl_2N_3O_4$: C, 55.06; H, 4.39; N, 9.63%. Found: C, 54.89; H, 4.58; N, 9.45%.

2-{2-[1-(3-Chloro-4-methoxyphenyl)-5-oxo-3-pyrrolidinyl]-1*H*-benzimidazol-1-yl}acetic acid (17). A mixture of **16** (0.44 g, 1 mmol) and 5% aqueous NaOH solution (10 mL) was heated at reflux for 1 min. Afterwards conc. acetic acid was added to pH 6. The crystalline precipitate was filtered, and washed with water, recrystallized from propan-2-ol to afford white solid, yield 0.36 g (90%); mp 275–277 °C; IR (KBr): ν_{max} 3347 (OH), 1720, 1678 (CO), 1610 (CN) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.87–3.05 (m, 2H, $COCH_2$), 3.84 (s, 3H, OCH_3), 4.01–4.28 (m, 3H, CHCN, NCH_2CH), 5.18 (s, 2H, CH_2COOH), 7.14–7.87 (m, 7H, aromatic H), 13.39 (br s, 1H, OH); ^{13}C NMR (101 MHz, DMSO-d_6): δ 28.5 (CH), 37.4 (CH_2CO), 44.5 (NCH_2CO), 52.1 (CH_2COOH), 56.3 (OCH_3), 110.1, 112.7, 118.8, 119.5, 120.8, 121.4, 121.8, 122.3, 132.9, 135.9, 141.7, 151.2, 155.4 (aromatic C), 169.9, 171.7 (CO); HRMS (ESI): *m/z* calcd for $C_{20}H_{18}ClN_3O_4+H^+$: 400.1059 [M+H^+]; found: 400.1056; *Anal.* Calcd for $C_{20}H_{18}ClN_3O_4$: C, 60.08; H, 4.54; N, 10.51%. Found: C, 60.37; H, 4.45; N, 10.72%.

3-{2-[1-(3-Chloro-4-methoxyphenyl)-5-oxo-3-pyrrolidinyl]-1*H*-benzimidazol-1-yl}propanoic acid (18). A mixture of **2** (3.08 g, 9 mmol), acrylic acid (1.30 g, 1.23 mL, 18 mmol) and 50% acetic acid (30 mL) was heated at reflux for 42 h and diluted with water (30 mL). After cooling, the precipitate was filtered off and washed with water, recrystallized from propan-2-ol to afford white solid, yield 2.15 g (58%); mp 228–229 °C; IR (KBr): ν_{max} 3360 (OH), 1697, 1650 (CO), 1608 (CN) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.81 (t, J = 2.8 Hz, 2H, $COCH_2$), 2.91–3.09 (m, 2H, CH_2COOH), 3.84 (s, 3H, OCH_3), 4.11–4.32 (m, 3H, CHCN, NCH_2CH), 4.50 (t, J = 7.0 Hz, 2H, NCH_2CH_2), 7.12–7.90 (m, 7H, aromatic H), 12.27 (s, 1H, OH); ^{13}C NMR (101 MHz, DMSO-d_6): δ 28.4 (CH), 33.9 (CH_2COOH), 37.8 (CH_2CO), 40.2(NCH_2CH_2), 52.3 (NCH_2CH), 56.3 (OCH_3), 110.5, 112.7, 118.8, 119.4, 120.8, 121.4, 121.7, 122.2, 132.9, 135.0, 142.1, 151.1, 155.2 (aromatic C), 172.1, 172.4 (CO); *Anal.* Calcd for $C_{21}H_{20}ClN_3O_4$: C, 60.95; H, 4.87; N, 10.15%. Found: C, 60.74; H, 4.71; N, 10.05%.

3-(1*H*-Benzimidazol-2-yl)-4-(3-chloro-4-methoxyphenylamino)butanoic acid (19). A mixture of compound **2** (1.71 g, 5 mmol) and 20% aqueous NaOH solution (20 mL) was heated at reflux for 6 h, then cooled to room temperature and acidified with acetic acid to pH 6. The precipitated product was filtered off, washed with water, and purified by dissolving the solid in a sodium hydroxide solution (5%), filtering and acidifying the filtrate with acetic acid (30%). The precipitate was filtered off, washed with water, recrystallized from propan-2-ol to afford white solid, yield 1.14 g (63%); mp 134–135 °C; IR

(KBr): ν_{max} 3361 (OH) 3166, 3061 (NH), 1613 (CO), 1580 (CN) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.72–2.92 (m, 2H, CH_2COOH), 3.20–3.54 (m, 3H, NHCH_2, CH_2CHCH_2), 3.70 (s, 3H, OCH_3), 5.70 (br. s, 1H, $NHCH_2$), 6.54–7.52 (m, 7H, aromatic H), 12.39 (br. s, 2H, NH, OH); ^{13}C NMR (101 MHz, DMSO-d_6): δ 35.5 (CH_2CHCH_2), 36.1 (CH_2COOH), 47.2 ($NHCH_2$), 56.6 (OCH_3), 111.4, 113.4, 114.7, 121.2, 121.9, 143.5, 145.7, 156.1 (aromatic C), 173.4 (CO); HRMS (ESI): *m/z* calcd for $C_{18}H_{18}ClN_3O_3+H^+$: 360.1109 [$M+H^+$]; found: 360.1110; *Anal.* Calcd for $C_{18}H_{18}ClN_3O_3$: C, 60.09; H, 5.04; N, 11.68%. Found: C, 60.33; H, 5.17; N, 11.76%.

3-(1*H*-Benzimidazol-2-yl)-4-(3-chloro-4-methoxyanilino)butanohydrazide (20). A mixture of butanoic acid **19** (1.08 g, 3 mmol) and hydrazine monohydrate (0.45 g, 0.44 mL, 9 mmol) was refluxed in toluene (15 mL) for 28 h. The reaction mixture was cooled, the precipitated compound was filtered off, washed with propan-2-ol and recrystallized from propan-2-ol to afford white solid, yield 0.75 g (67%); mp 221–222 °C; IR (KBr): ν_{max} 3311, 3175, 3140, 3115 (NH_2, 3NH), 1678 (CO), 1610 (CN) cm^{-1}; ^1H NMR (700 MHz, DMSO-d_6): δ 2.54–2.66 (m, 2H, CH_2CO), 3.24–3.45 (m, 2H, NHCH_2), 3.63–3.69 (m, 1H, CH_2CHCH_2), 3.70 (s, 3H, OCH_3), 4.21 (br. s, 2H, NH_2), 5.62 (t, J = 5.7 Hz, 1H, $NHCH_2$), 6.53–7.57 (m, 7H, aromatic H), 9.08 (s, 1H, CONH), 12.24 (s, 1H, NH); ^{13}C NMR (176 MHz, DMSO-d_6): δ 35.4 (CH_2CHCH_2), 35.7 (CH_2CO), 47.2 (NCH_2CO), 56.6 (OCH_3), 111.0, 111.4, 113.4, 114.7, 118.3, 120.8, 121.5, 121.9, 134.2, 143.2, 143.6, 145.6, 156.3 (aromatic C), 169.9 (CO); *Anal.* Calcd for $C_{18}H_{20}ClN_5O_2$: C, 57.83; H, 5.39; N, 18.73%. Found: C, 57.66; H, 5.51; N, 18.68%.

General procedure for the synthesis of hydrazones 21–24.

A mixture of hydrazide **20** (0.37 g, 1 mmol) and 1.1 mmol of the corresponding aldehyde in propan-2-ol (20 mL) was heated under reflux for 5 h. The reaction mixture was cooled, the precipitate was filtered off, washed with propan-2-ol and dried.

3-(1*H*-Benzimidazol-2-yl)-4-(3-chloro-4-methoxyanilino)-*N*′-(2-furylmethylidene)butanohydrazide (21) was prepared from **20** and 2-furaldehyde. Recrystallized from propan-2-ol to afford white solid, yield 0.32 g (71%); mp 109–110 °C; IR (KBr): ν_{max} 3196, 3119, 3055 (3NH), 1671 (CO), 1622, 1581 (CN) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6), (*Z/E* isomeric mixture, 60/40): δ 2.64–3.15 (m, 2H, CH_2CO), 3.33–3.53 (m, 2H, NHCH_2), 3.70 (s, 2H, OCH_3), 3.94–4.30 (m, 1H, CH_2CHCH_2), 5.63–5.75 (m, 1H, $NHCH_2$), 6.54–7.97 (m, 10H, aromatic H), 8.01 (s, 0.6H, N=CH), 8.51 (s, 0.4H, N=CH), 11.23 (s, 0.6H, CONH), 11.43 (s, 0.4H, CONH), 12.27 (s, 1H, NH); *Anal.* Calcd for $C_{23}H_{22}ClN_5O_3$: C, 61.13; H, 4.91; N, 15.50%. Found: C, 61.05; H, 4.73; N, 15.61%.

3-(1*H*-Benzimidazol-2-yl)-4-(3-chloro-4-methoxyanilino)-*N*′-[(2-thienyl)methylidene]butanohydrazide (22). was prepared from **20** and 2-thiophenecarbaldehyde. Recrystallized from propan-2-ol to afford

white solid, yield 0.28 g (60%); mp 139–141 °C; IR (KBr): ν_{max} 3191, 3061, 3101 (3NH), 1678 (CO), 1611, 1596 (2CN) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6), (*Z/E* isomeric mixture, 60/40): δ 2.58–3.13 (m, 2H, CH_2CO), 3.16–3.32 (m, 1.2H, NH*CH*$_2$), 3.41–3.52 (m, 1H, CH_2*CH*CH_2), 3.66–3.78 (m, 0.8H, NH*CH*$_2$), 3.70 (s, 3H, OCH_3), 5.60–5.79 (m, 1H, *NH*CH_2), 6.52–7.68 (m, 10H, aromatic H), 8.15 (s, 0.6H, N=CH), 8.33 (s, 0.4H, N=CH), 11.28 (s, 0.6H, CONH), 11.47 (s, 0.4H, CONH), 12.30 (s, 1H, NH); *Anal.* Calcd for $C_{23}H_{22}ClN_5O_2S$: C, 59.03; H, 4.74; N, 14.97%. Found: C, 59.11; H, 4.86; N, 14.95%.

3-(1*H*-Benzimidazol-2-yl)-4-(3-chloro-4-methoxyanilino)-*N*′-[(4-chlorophenyl)methylidene]butanohydrazide (23) was prepared from **20** and 4-chlorobenzaldehyde. Recrystallized from propan-2-ol to afford white solid, yield 0.35 g (71%); mp 136–138 °C; IR (KBr): ν_{max} 3274, 3190, 3157 (3NH), 1661 (CO), 1625, 1608 (2CN) cm^{-1}; ^1H NMR (400 MHz, DMSO-*d*$_6$), (*Z/E* isomeric mixture, 60/40): δ 2.64–3.21 (m, 2H, CH_2CO), 3.25–3.30 (m, 0.8H, NH*CH*$_2$), 3.42–3.53 (m, 1H, CH_2*CH*CH_2), 3.70 (s, 3H, OCH_3), 3.72–3.82 (m, 1.2H, NH*CH*$_2$), 5.60–5.78 (m, 1H, *NH*CH_2), 6.54–7.99 (m, 11H, aromatic H), 8.11 (s, 0.4H, N=CH), 8.72 (s, 0.6H, N=CH), 11.33 (s, 0.6H, CONH), 11.56 (s, 0.4H, CONH), 12.28 (s, 1H, NH); HRMS (ESI): *m/z* calcd for $C_{25}H_{23}Cl_2N_5O_2$+H^+: 496.1302 [M+H^+]; found: 496.1305; *Anal.* Calcd for $C_{25}H_{23}Cl_2N_5O_2$: C, 60.49; H, 4.67; N, 14.11%. Found: C, 60.73; H, 4.76; N, 13.95%.

3-(1*H*-Benzimidazol-2-yl)-4-(3-chloro-4-methoxyanilino)-*N*′-[(2-chloro-5-nitrophenyl)methylidene]-butanohydrazide (24) was prepared from **20** and 2-chloro-5-nitrobenzaldehyde. Recrystallized from propan-2-ol to afford white solid, yield 0.31 g (57%); mp 211–212 °C; IR (KBr): ν_{max} 3276, 3214, 3143 (3NH), 1676 (CO), 1620, 1609 (2CN) cm^{-1}; ^1H NMR (700 MHz, DMSO-*d*$_6$), (*Z/E* isomeric mixture, 60/40): δ 2.81–3.16 (m, 2H, CH_2CO), 3.35–3.54 (m, 1H, CH_2*CH*CH_2), 3.71 (s, 3H, OCH_3), 3.73–3.81 (m, 2H, NH*CH*$_2$), 5.67–5.77 (m, 1H, *NH*CH_2), 6.54–8.56 (m, 10H, aromatic H), 8.59 (s, 0.4H, N=CH), 8.62 (s, 0.6H, N=CH), 11.68 (s, 0.6H, CONH), 11.98 (s, 0.4H, CONH), 12.31 (s, 0.6H, NH), 12.33 (s, 0.4H, NH); *Anal.* Calcd for $C_{25}H_{22}Cl_2N_6O_4$: C, 55.46; H, 4.10; N, 15.52%. Found: C, 55.27; H, 4.15; N, 15.41%.

REFERENCES

1. (a) Y. Özkay, Y. Tunalı, H. Karaca, and İ. Işıkdağ, *Eur. J. Med. Chem.*, 2010, **45**, 3293; (b) C. Kuş, H. Göker, G. Ayhan, R. Ertan, N. Altanlar, and A. Akin, *Il Farmaco,* 1996, **51**, 413; (c) H. Göker, C. Kus, and U. Abbasoglu, *Arch. Pharm.,* 1995, **328**, 425; (d) N. C. Desai, D. D. Pandya, V. V. Joshi, K. M. Rajpara, H. V. Vaghani, and H. M. Satodiya, *Med. Chem. Res.*, 2012, **21**, 4463; (e) K. F. Ansari and C. Lal, *Eur. J. Med. Chem.*, 2009, **44**, 4028.
2. (a) M. P. Nitin and P. T. Gowda, *Int. Res. J. Pharm.*, 2012, **3**, 189–194; (b) W. A. Maxwell and G. Brody, *Appl. Microbiol.*, 1971, **21**, 944; (c) C. J. Goin and V. W. Mayer, *Mutat. Res.*, 1995, **343**, 185; (d) Y. B. Bai, A. L. Zhang, J. J. Tang, and J. M. Gao, *J. Agric. Food. Chem.*, 2013, **61**, 2789.

3. (a) M. Bretner, A. Baier, K. Kopańska, A. Najda, A. Schoof, M. Reinholz, A. Lipniacki, A. Piasek, T. Kulikowski, and P. Borowski, *Antivir. Chem. Chemother.*, 2005, **16**, 315; (b) M. Tonelli, G. Paglietti, V. Boido, F. Sparatore, F. Marongiu, E. Marongiu, P. L. Colla, and R. Loddo, *Chem. Biodivers.*, 2008, **5**, 2386; (c) S. Liu, C.A. Nelson, L. Xiao, L. Lu, P. P. Seth, D. R. Davis, and C. H. Hagedorn, *Antiviral Res.*, 2011, **89**, 54.
4. (a) K. Garg, Y. Bansal, G. Bansal, and R. K. Goel, *Med. Chem. Res.*, 2014, **23**, 2690; (b) G. Navarette-Vázquez, L. Yépez, A. Hernández-Campos, A. Tapia, F. Hernández-Luis, R. Cedillo, J. González, A. Martínez-Fernández, M. Martínez-Grueiro, and R. Castillo, *Bioorg. Med. Chem.*, 2003, **11**, 4615; (c) J. Valdez, R. Cedillo, A. Hernández-Campos, L. Yépez, F. Hernández-Luis, G. Navarrete-Vázquez, A. Tapia, R. Cortés, M. Hernández, and R. Castillo, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 2221; (d) A. T. Mavrova, D. Vuchev, K. Anichina, and N. Vassilev, *Eur. J. Med. Chem.*, 2010, **45**, 5856.
5. (a) K. Kubo, Y. Kohara, Y. Yoshimura, Y. Inada, Y. Shibouta, Y. Furukawa, T. Kato, K. Nishikawa, and T. Naka, *J. Med. Chem.*, 1993, **36**, 2343; (b) A. Jain, R. Sharma, and S. C. Chaturvedi, *Med. Chem. Res.*, 2013, **22**, 4622.
6. (a) S. M. Sondhi, N. Singh, A. Kumar, O. Lozach, and L. Meijer, *Bioorg. Med. Chem.*, 2006, **14**, 3758; (b) M. Gaba, S. Singh, and C. Mohan, *Eur. J. Med. Chem*., 2014, **76**, 494.
7. (a) K. Starcevic, M. Kralj, K. Ester, I. Sabol, M. Grce, K. Pavelic, and G. Karminski-Zamola, *Bioorg. Med. Chem.*, 2007, **15**, 4419; (b) M. M. Ramla, M. A. Omar, A. M. El-Khamry, and H. I. El-Diwani, *Bioorg. Med. Chem.*, 2006, **14**, 7324.
8. (a) S. R. Zheng, S. L. Cai, J. B. Tan, J. Fan, and W. G. Zhang, *Inorg. Chem. Commun*., 2012, **100**, 1275; (b) J. Kulhanek and F. Bureš, *Beilstein J*, *Org.chem*., 2012, **8**, 25.
9. K. Mickeviciene, A. Voskiene, and V. Mickevicius, *Res. Chem. Intermed.*, 2014, **40**, 1619.
10. V. Mickevicius, R. Vaickelioniene, I. Jonuskiene, G. Mikulskiene, and K. Kantminiene, *Monatsh. Chem.*, 2009, **140**, 1513.
11. M. Mickevicius, Z. Beresnevicius, V. Mickevicius, and G. Mikulskiene, *Heteroatom Chem*., 2006, **17**, 47.
12. A. K. Zafer, T. Z. Gulhan, O. Ahmet, and R. Gilbert, *Eur. J. Med. Chem.*, 2008, **43**, 155.
13. I. Chopra, C. Schofield, M. Everett, A. O'Neill, K. Miller, M. Wilcox, J. M. Frère, M. Dawson, L. Czaplewski, U. Urleb, and P. Courvalin, *Lancet. Infect. Dis.,* 2008, **8**, 133.
14. M. Alp, A. H. Göker, and N. Altanlar, *Turk. J. Chem.*, 2014, **38**, 152.