Tetrahedron 71 (2015) 9202–9215

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Synthesis of new 2-aminoimidazolones with antiproliferative activity via base promoted amino-β-lactam rearrangement

Tonko Dražić [a,*], Katarina Vazdar [a], Mario Vazdar [a], Marijana Đaković [b], Ana-Matea Mikecin [a], Marijeta Kralj [a], Martina Malnar [a], Silva Hećimović [a], Ivan Habuš [a]

[a] Ruđer Bošković Institute, Bijenička c. 54, HR-10002 Zagreb, Croatia
[b] Faculty of Science, Horvatovac 102a, HR-10002 Zagreb, Croatia

ARTICLE INFO

Article history:
Received 24 July 2015
Received in revised form 6 October 2015
Accepted 16 October 2015
Available online 19 October 2015

Keywords:
β-Lactam
Guanidine
2-Aminoimidazolone
Rearrangement
Antiproliferative activity

ABSTRACT

A facile and efficient transformation of amino-β-lactam guanidines to 2-aminoimidazolones is described. The base-promoted transformation proceeds in two steps, with the rearrangement of four-membered β-lactam ring to five-membered imidazolone and subsequent E1cB elimination and formation of double bond at the 4-position of imidazolone ring, which is supported with quantum chemical calculations. The benzoylaminoimidazolone and 2-aminoimidazolone products are obtained in high yields. The benzoylaminoimidazolone products show antiproliferative activity in HCT116 (colon carcinoma) and H460 (lung carcinoma) cell lines.

1. Introduction

β-Lactams are useful building blocks for the synthesis of a large number of organic molecules, such as α-amino acids, β-amino acids, their derived peptides, and other nitrogen containing heterocycles and open chain molecules.[1] Nucleophilic cleavage of the 2-azetidinone ring at the N1–C2 bond (Fig. 1) usually leads to the formation of β-amino acids.[2] However, it has been shown that methanolysis of an appropriately substituted β-lactam derivative with sodium or potassium methoxide results in the direct formation of a five-membered ring from the azetidinone ring. Using this approach, Alcaide et al. prepared cyclic enaminones,[3] as well as pyrrole derivatives.[4] On the other hand, Mehra et al. employed sodium methoxide for the preparation of thioxoimidazolidines, imidazolidin-2-ones and 4,5-dihydroimidazoles.[5,6]

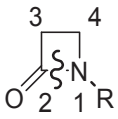


Fig. 1. β-Lactam ring N1–C2 bond cleavage.

In our investigation we introduced a guanidine group at the C3 position of the azetidinone ring due to its high nucleophilicity,[7–9] in order to promote the transformation from the four-membered azetidinone ring to a five-membered 2-aminoimidazolone ring. 2-Aminoimidazoles are an important group of heterocyclic compounds and their structural motif is present in many natural alkaloids isolated mainly from marine organisms.[10–13] Naturally occurring aminoimidazoles, as well as their synthetic analogues, possess a wide range of biological activity. For example, marine natural products isolated from sponges have anti HIV, antifungal and antimicrobial activity.[14] 2-Aminoimidazole derivatives are inhibitors of β-secretase,[15–18] a key enzyme in the pathogenesis of Alzheimer's disease. 2-Aminoimidazole amino acids are inhibitors of the Binuclear Manganese Metalloenzyme Human Arginase I, an enzyme that catalyzes the hydrolysis of L-arginine to L-ornithine and urea.[19] 2-Aminoimidazolones, an important group of 2-aminoimidazoles, together with 2-aminothiazolone derivatives possess anticancer activity.[20]

Methods for the synthesis of 2-aminoimidazoles, including 2-aminoimidazolones, were described in the middle of the 20th century,[21,22] however the most progress was achieved after the 1980s, when the 2-aminoimidazole and 2-aminoimidazolone containing alkaloids from marine sponges were first discovered and isolated. Most of these methods have been developed and applied in the total synthesis of marine alkaloids.[23–33] Some of the methods include cyclocondensation of α-halocarbonyl compounds

* Corresponding author. E-mail address: tdrazic@irb.hr (T. Dražić).

http://dx.doi.org/10.1016/j.tet.2015.10.048
0040-4020/

with *N*-protected guanidine,[23–25] α-aminocarbonyl derivatives with cyanamide,[26] or *S*-methylisothiourea with β-aminodiacetals.[27] Furthermore, 2-aminoimidazolones can be obtained from 2-thiohydantoin.[20,29,32,33] Despite the existence of numerous methods in literature for the synthesis of 2-aminoimidazoles and 2-aminoimidazolones, direct preparation from a four-membered ring has not been reported yet.

In this paper we describe a novel method for the preparation of benzoyl-2-aminoimidazolone and 2-aminoimidazolone derivatives (Fig. 2) directly from guanidine-β-lactams. We propose the reaction mechanism based on experimental evidence and support it with quantum-chemical calculations. Furthermore, we determined antiproliferative activity of new compounds on two human tumor cell lines and tested a potential inhibitory activity of new compounds against β-secretase.

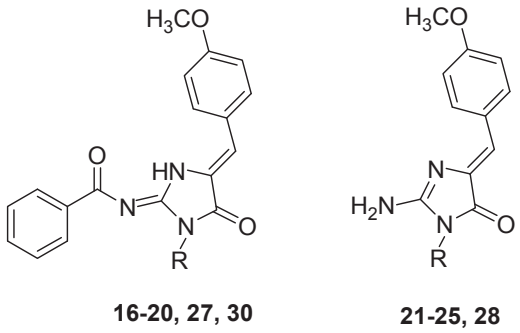


Fig. 2. Novel benzoylaminoimidazolones **16**–**20**, **27** and **30** and 2-aminoimidazolones **21**–**25** and **28**.

2. Results and discussion

2.1. Synthesis

Enantiomerically pure *trans*-3-amino-β-lactams (3*R*,4*R*)-**1a** and (3*S*,4*S*)-**1b** (Fig. 3) were synthesized applying the chiral ester enolate–imine condensation according to the procedure described by Ojima et al.[34] and Poljak et al.[35] (1*R*,2*S*,5*R*)-(−)-menthol was used as chiral auxiliary for the synthesis of (3*R*,4*R*)-**1a** enantiomer, whereas for the synthesis of (3*S*,4*S*)-**1b** enantiomer (1*S*,2*R*,5*S*)-(+)-menthol was used.

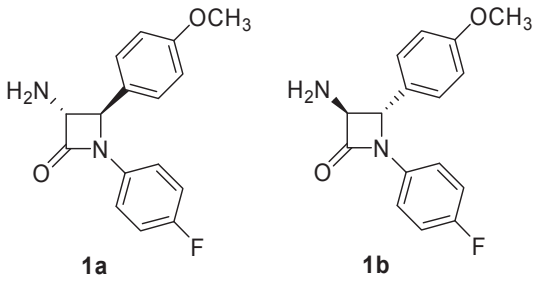


Fig. 3. Structure of *trans*-3-amino-β-lactams (3*R*,4*R*)-**1a** and (3*S*,4*S*)-**1b**.

Thioureas **2**–**8** were obtained from benzoyl isothiocyanate and corresponding amines in 60–90% yield. β-Lactam guanidines **9a**–**13** were synthesized in high yields (60–87%) from amino-β-lactam **1a** and thioureas **2**–**6** by applying $HgCl_2$-promoted guanylation described by Cunha et al. (Scheme 1),[36] when **1a** was reacted with thioureas **7** and **8**, the expected guanidines **14** and **15** were not detected.

The treatment of guanidines **9**–**13** with 2.0 equiv of K_2CO_3 in methanol at room temperature resulted in almost quantitative formation of benzoylaminoimidazolones **16**–**20** and 2-aminoimidazolones **21**–**25** (Scheme 2).

In order to confirm the structure of obtained products and determine the stereochemistry around the newly formed double bond, as well as the position of the benzoyl group, we prepared single crystals of 2-aminoimidazolone **22** (Fig. 4) and intermediate **26** (Fig. 5, vide infra). The newly formed double bond in **22** was of *Z*-configuration, whereas the configuration around the guanidine double bond in **26** was *E*. Furthermore, only one isomer can be detected in the NMR spectra of all compounds.

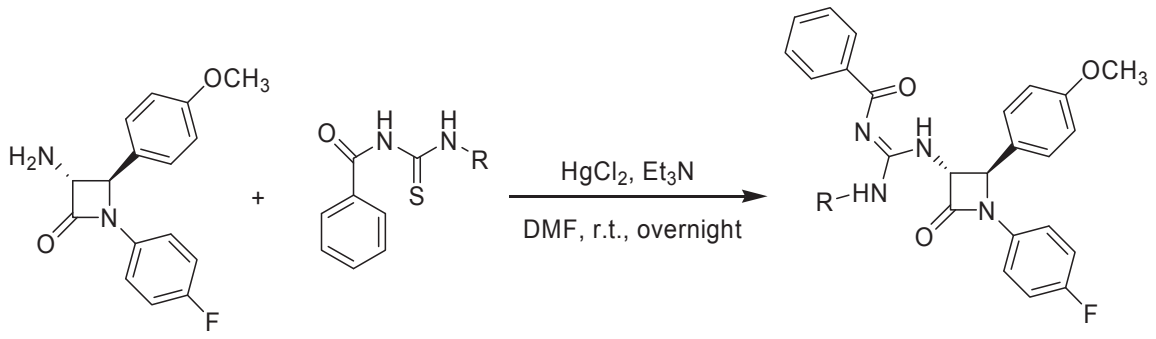
The formation of imidazolone derivatives by the treatment of the corresponding guanidines with K_2CO_3 in methanol occurs via N1–C2 amidolysis of β-lactam ring and rearrangement to five-membered imidazolone ring. 2-Aminoimidazolones are formed as a consequence of benzoyl group cleavage in the presence of base.

When thioureas **7** and **8**, containing electron withdrawing groups (i.e., cyano and nitro) were subjected to guanylation with β-lactam **1a**, the expected guanidines **14** and **15** could not be isolated. In an attempt to obtain β-lactam guanidine **14**, imidazolone intermediate **26** was isolated (Scheme 3). The structure of intermediate **26** was confirmed by X-ray crystallography (Fig. 5). Guanidine **14** undergoes the rearrangement to intermediate **26** in the given reaction conditions presumably due to electron withdrawing effect of cyano group on the side chain phenyl. However, in the guanylation reaction conditions, the presumed imidazolone product **27** was not formed. When intermediate **26** was treated with K_2CO_3 in methanol, final products **27** and **28** were obtained (90% and ~1%, respectively). Subjecting intermediate **26** to purification by silica gel column chromatography also led to the formation of product **27**, but then no product **28** was detected.

Guanylation reaction of thiourea **8** and β-lactam **1a** afforded a mixture of several compounds including the final product **24** and presumably five-membered ring intermediate **29** (detected by ^1H NMR spectroscopy) (Scheme 4). Guanidine **15** was not detected upon the reaction completion and pure imidazolone intermediate **29** could not be isolated and fully characterized. The treatment of the mixture obtained in the guanylation reaction with K_2CO_3 resulted in the formation of benzoylaminoimidazolone **30** in 62% yield. However, the expected corresponding deprotected 2-aminoimidazolone was not detected.

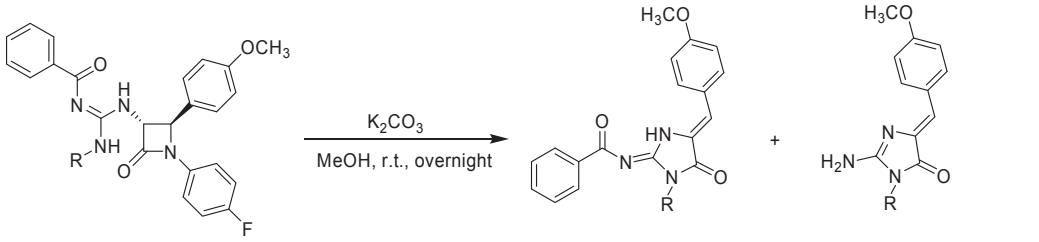
The influence of K_2CO_3 ratio on the formation of benzoylaminoimidazolone and 2-aminoimidazolone products was further investigated. We wanted to determine if reaction required stoichiometric base, as well as if the amount of base influences the ratio of benzoyl and hydrolyzed product. For this purpose guanidine **9a** was treated with different amounts of K_2CO_3 (0.1–20 equiv) in MeOH overnight at room temperature (Table 1). When the amount of K_2CO_3 was 0.5 equiv or higher, the product ratio and the reaction time remained unchanged. However, when 0.1 equiv of K_2CO_3 was used, the reaction proceeded much slower giving benzoylaminoimidazolone **16** in lower yield (75%) and 2-aminoimidazolone **21** was not detected (Table 1). Despite the lower reaction rate when 0.1 equiv of K_2CO_3 was applied, intermediate **31** (Scheme 5, vide infra) could not be detected. These results show that K_2CO_3 can act in a catalytic as well as stoichiometric quantity and the ratio of final products remains unchanged when the amount of K_2CO_3 is above 0.1 equiv.

In order to further elucidate the role of K_2CO_3, we used acetonitrile as a solvent instead of methanol to exclude formation of potassium methoxide. The reaction was carried out with β-lactam **1a** and thiourea **2** in acetonitrile following the general procedure for the preparation of benzoylaminoimidazolones and gave a mixture of intermediate **31** (22%) and product **16** (41%) (Scheme 5). Intermediate **31** was isolated as a mixture of two isomers which



1a	2-8	9-15	yield
	2, R = Ph	**9a**, R = Ph	60%
	3, R = 4-F-C_6H_4	**10**, R = 4-F-C_6H_4	77%
	4, R = 4-CH_3O-C_6H_4	**11**, R = 4-CH_3O-C_6H_4	78%
	5, R = 4-CH_3-C_6H_4	**12**, R = 4-CH_3-C_6H_4	78%
	6, R = cyclohexyl	**13**, R = cyclohexyl	87%
	7, R = 4-NC-C_6H_4	**14**, R = 4-NC-C_6H_4	/
	8, R = 4-O_2N-C_6H_4	**15**, R = 4-O_2N-C_6H_4	/

Scheme 1. Synthesis of guanidines **9**–**13** from β-lactam **1a** and thioureas **2**–**8**.



9-13	16-20	yield	21-25	yield
9a, R = Ph	**16**, R = Ph	82%	**21**, R = Ph	17%
10, R = 4-F-C_6H_4	**17**, R = 4-F-C_6H_4	83%	**22**, R = 4-F-C_6H_4	17%
11, R = 4-CH_3O-C_6H_4	**18**, R = 4-CH_3O-C_6H_4	92%	**23**, R = 4-CH_3O-C_6H_4	8%
12, R = 4-CH_3-C_6H_4	**19**, R = 4-CH_3-C_6H_4	69%	**24**, R = 4-CH_3-C_6H_4	15%
13, R = cyclohexyl	**20**, R = cyclohexyl	66%	**25**, R = cyclohexyl	26%

Scheme 2. Synthesis of benzoylaminoimidazolones **16**–**20** and 2-aminoimidazolones **21**–**25**.

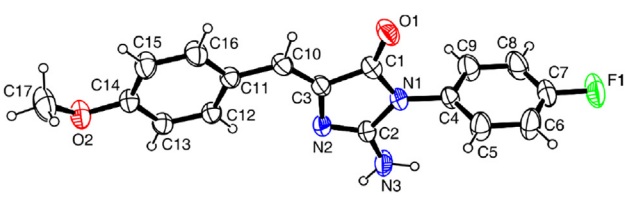


Fig. 4. Molecular structure of **22** with the atom numbering scheme.

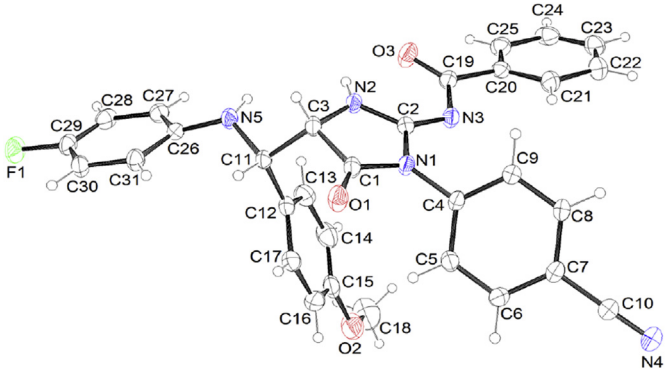
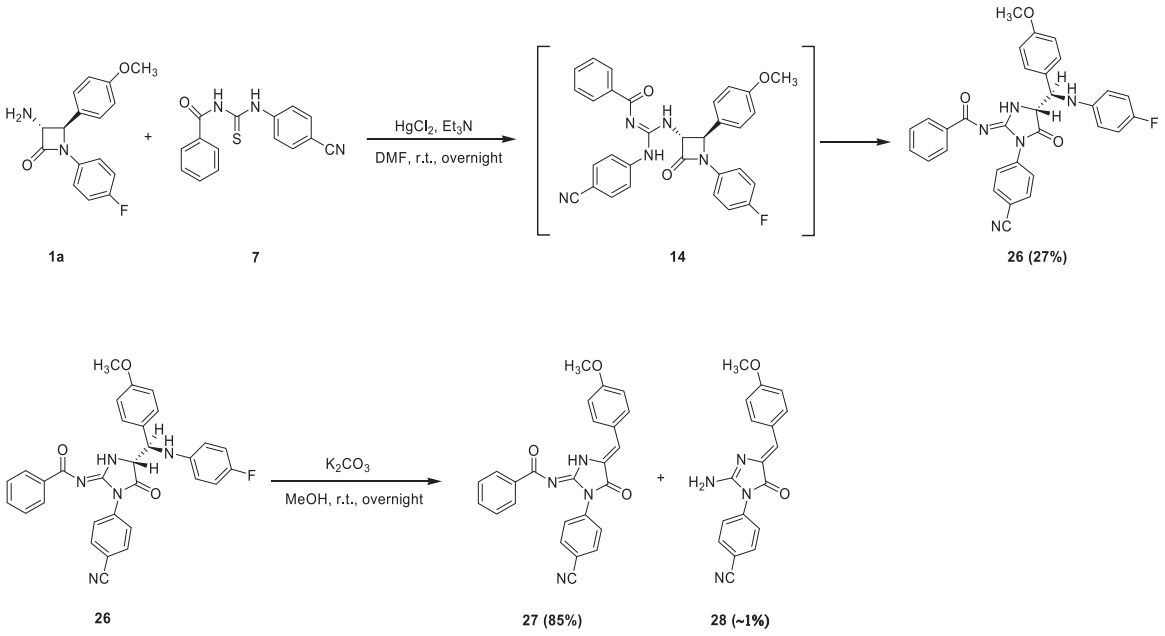


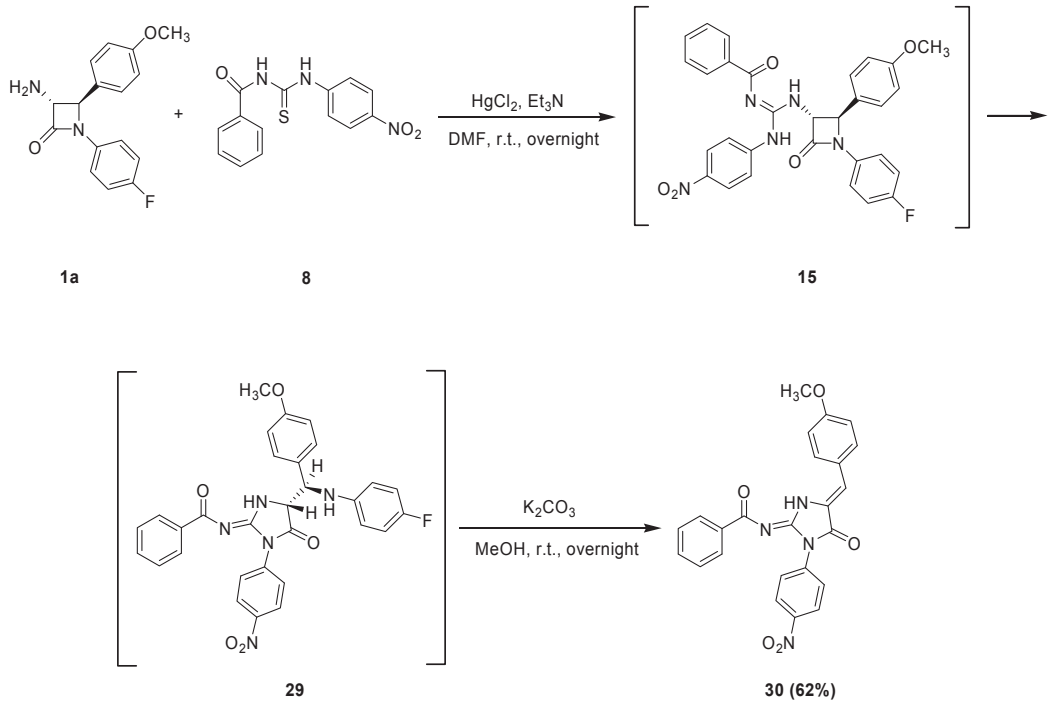
Fig. 5. Molecular structure of **26** with the atom numbering scheme.

could not be separated and their configuration could not be unequivocally determined.

To exclude the possibility that the stereochemistry of the starting β-lactam guanidine influences the reaction course, or intermediate and product structures, we synthesized guanidine **9b** from (3*S*,4*S*)-enantiomer of amino-β-lactam (**1b**) (Fig. 3) and subjected it to K_2CO_3 promoted transformation. The reaction resulted in the formation of benzoylaminoimidazolone **16** and 2-aminoimidazolone **21** with identical yields as in the reaction with **9a** (82% and 17%, respectively). The above results strongly suggest that the transformation reaction of β-lactam guanidine to five-membered imidazolones is independent of β-lactam, imidazolone or side chain stereochemistry.



Scheme 3. Formation of intermediate **26** from amino-β-lactam **1a** and thiourea **7** and its subsequent transformation to final products **27** and **28**.



Scheme 4. Synthesis of benzoylaminoimidazolone **30**.

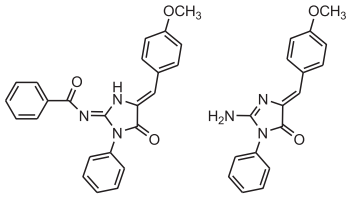
2.2. Reaction mechanism

In order to further understand the β-lactam reactivity and to elucidate the formation of described products, we propose the reaction mechanism shown in Scheme 6. To support the idea, we performed quantum chemical calculations with model compounds denoted as **M-1**-**M-7, M-3′**-**M-7′**, **M-TS$_1$**, **M-TS-isomerization**, **M-TS$_2$** and **M-TS$_2$′** (see Computational Details). Gibbs free energy differences, along with all calculated energies and Gibbs free energy data, are given in Table 2. All data correspond to experimental conditions where reactions are performed in methanol at 298 K.

In the first step, model guanidine β-lactam **M-1** is deprotonated by a base forming anion **M-2**. The anion **M-2** undergoes an intramolecular nucleophilic attack of the guanidinium nitrogen atom toward carbonyl group of the β-lactam ring forming five-membered ring in imidazolone anion **M-3**. Model quantum chemical calculations suggest that the reaction occurs via a bicyclic transition state **M-TS$_1$** presented in Fig. 6.

Fig. 6 shows formation of the five-membered ring and simultaneous cleavage of the four-membered β-lactam ring via the transition state **M-TS$_1$**. Inspection of the selected C—N bonds reveals a shortening of the distance between the guanidinium nitrogen

Table 1
The influence of K_2CO_3 amount on the transformation reaction of guanidine **9a**[a]

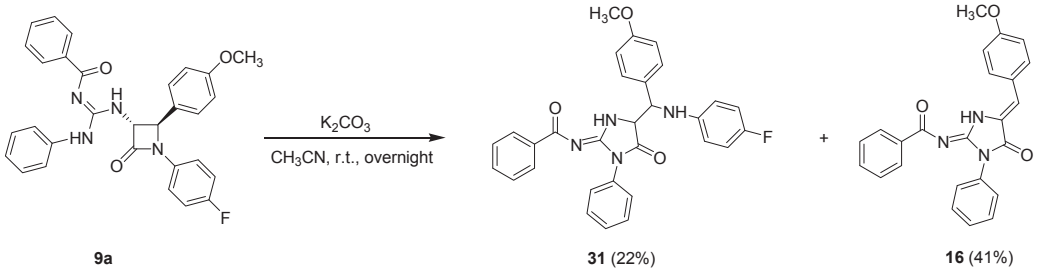


K_2CO_3 (equiv)	**16** yield (%)	**21** yield (%)
0.1[b]	75	—
0.5	88	10
1	92	7
20	90	8

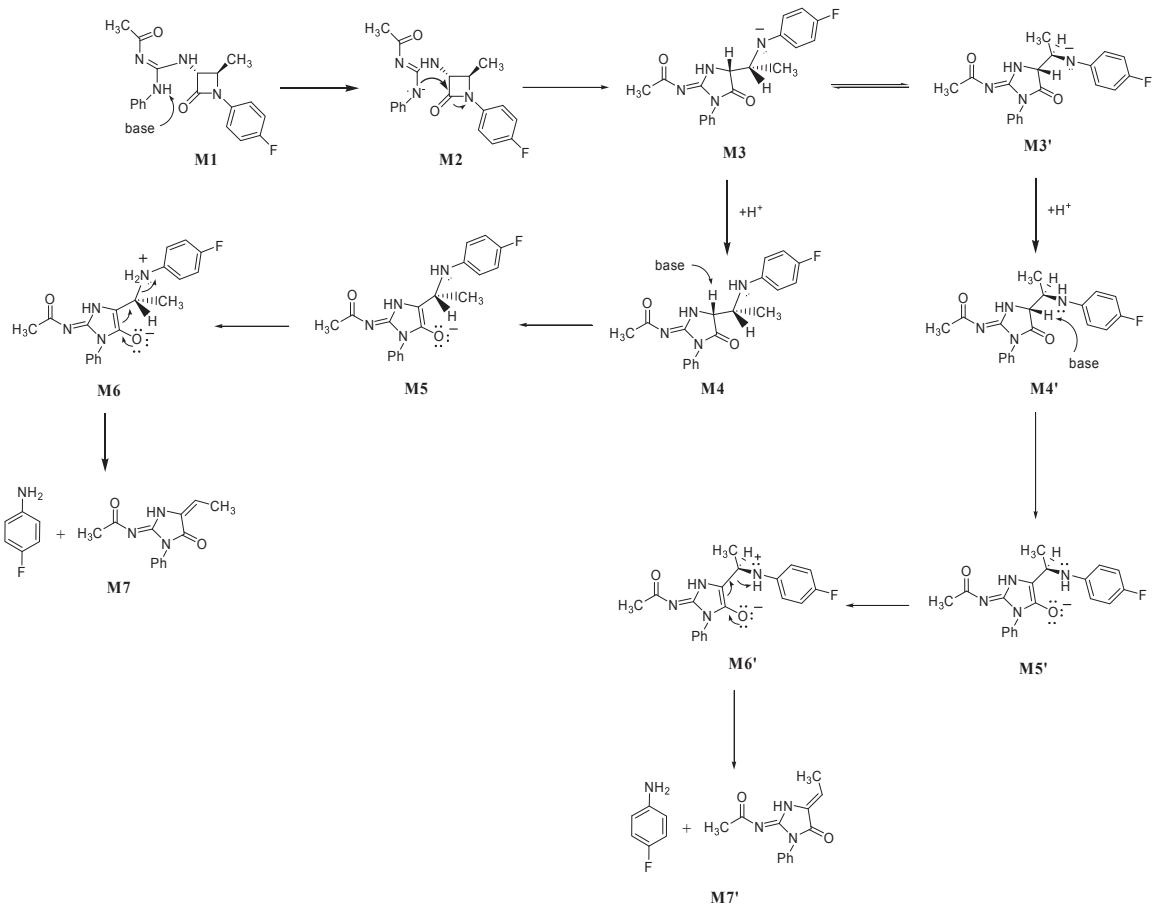
[a] The transformation reaction of guanidine **9a** (30 mg, 0.06 mmol) proceeded in the presence of indicated amount of K_2CO_3 in MeOH overnight at rt.
[b] Reaction proceeded for 4 days.

atom and the carbon atom of the β-lactam carbonyl group from 2.928 Å to 1.386 Å and a simultaneous cleavage of the corresponding C–N bond of the β-lactam ring from 1.368 Å to 2.881 Å via the transition state **M-TS$_1$**. The free energy activation barrier for the reaction is 16.9 kcal mol^{-1} whereas the barrier for the reverse reaction is 11.2 kcal mol^{-1}. It has been shown that β-lactam ring could also be specifically opened with methoxide (formed in the mixture of K_2CO_3 and methanol) to give methyl ester prior to the ring rearrangement.[5,6] However, this pathway could not be confirmed in our model conditions and seems unlikely. This is further corroborated by experimental modifications which demonstrate the formation of the same product regardless of the base used (i.e., Et_3N and K_2CO_3).

By monitoring the rotation around the C2–C3 bond of the imidazolone anion **M-3** (C1–C4 dihedral angle −78.1°) it is possible to obtain a stable isomeric structure of imidazolone **M-3′** (C1–C4 dihedral angle 54.7°), Fig. 7. The isomerization occurs via the



Scheme 5. Formation of intermediate **31** and final product **16** in CH_3CN.



Scheme 6. Proposed transformation of model β-lactam ring into a five-membered ring and subsequent elimination of aryl-amine group.

Table 2
Calculated total electronic energies, correction to Gibbs free energies, Gibbs free energies (in hartrees), Gibbs free energy differences and energy barriers (in kcal mol^{-1}) at 298 K for compounds **M-1**-**M-7**, **M-3′**-**M-7′**, **M-TS$_1$**, **M-TS$_{isomerization}$**, **M-TS$_2$**, **M-TS$_2$′** along the proposed reaction path

	B3LYP/6−31+G(d)	MP2/6−311++G(d,p)[a]	G_{corr}[a]	G	ΔG[b,c]	Energy barrier[c]
M-1	−1204.859021	−1201.794121	0.298585	−1201.495536	0.0	0.0
M-2	−1204.383361	−1201.319888	0.284546	−1201.035342	288.8	288.8
M-TS$_1$	−1204.349293	−1201.295367	0.286919	−1201.008448	305.7	16.9
M-3	−1204.370246	−1201.311973	0.285712	−1201.026261	294.5	−11.2
M-TS$_{isomerization}$	−1204.359731	−1201.299531	0.287750	−1201.011781	303.6	9.1
M-3′	−1204.370532	−1201.311115	0.285285	−1201.025831	294.7	−8.8
M-4	−1204.880454	−1201.823550	0.300704	−1201.522846	−17.1	−311.6
M-4′	−1204.877966	−1201.818676	0.300380	−1201.518296	−14.3	−309.0
M-5	−1204.394982	−1201.334172	0.286199	−1201.047973	280.9	298.0
M-5′	−1204.395988	−1201.335011	0.286580	−1201.048431	280.6	294.8
M-6	−1204.854220	−1201.794624	0.302231	−1201.492393	2.0	−278.9
M-6′	−1204.856162	−1201.796656	0.302440	−1201.494216	0.8	−279.7
M-TS$_2$	−1204.851183	−1201.784193	0.298953	−1201.485240	6.5	4.5
M-TS$_2$′	−1204.853322	−1201.786708	0.299327	−1201.487381	5.1	4.3
M-7	−1204.871200	−1201.795221	0.285881	−1201.509340	−8.7	−15.1
M-7′	−1204.874244	−1201.798497	0.286803	−1201.511694	−10.1	−15.3

[a] Calculated at the B3LYP/6−31+G(d) optimized geometry.
[b] With respect to reactant **1**.
[c] Calculated as the difference between Gibbs free energies for each step along the reaction path.

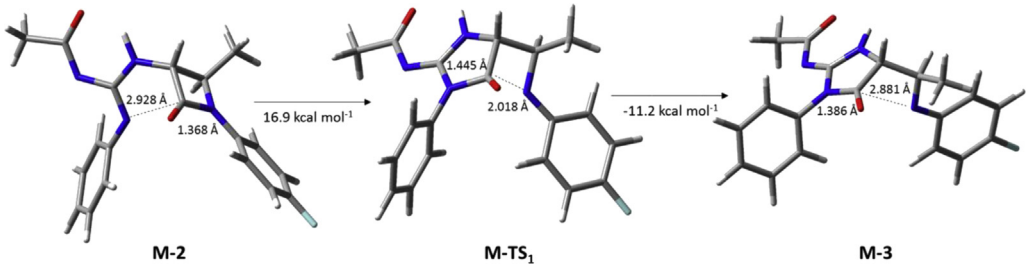


Fig. 6. Optimized structures of anion **M-2**, transition state **M-TS$_1$** and imidazolone anion **M-3** obtained at the SMD/B3LYP/6−31+G(d) level of theory with methanol as solvent.

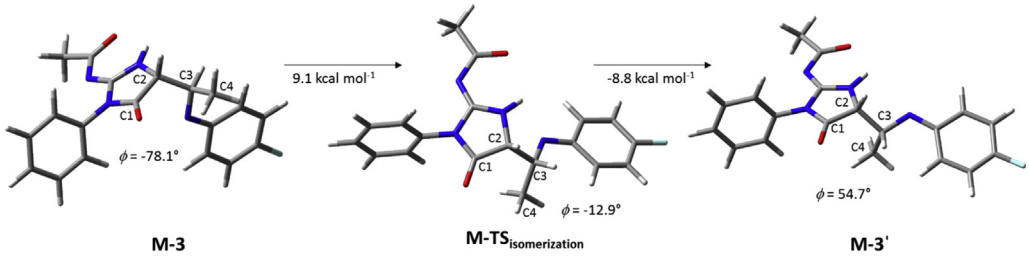


Fig. 7. Optimized structures of imidazolone anion **M-3**, transition state **M-TS$_{isomerization}$** and isomeric imidazolone anion **M-3′** obtained at the SMD/B3LYP/6−31+G(d) level of theory with methanol as solvent.

transition state **M-TS$_{isomerization}$**. The free energy activation barrier for the isomerization reaction is 9.1 kcal mol^{-1} whereas the barrier for the reverse reaction is 8.8 kcal mol^{-1}. The imidazolone **M-3** is only 0.2 kcal mol^{-1} more stable than the isomeric imidazolone **M-3′**and so is safe to assume that both of the structures are involved in the reaction pathway.

The second step of the reaction can proceed from both imidazolone anion **M-3** as well as the isomeric form **M-3′**. The imidazolone anions **M-3** and **M-3′** can be protonated to form intermediate **M-4** and **M-4′**, respectively. The intermediate **M-4** is by −17.1 kcal mol^{-1} more stable than the reactant **M-1** while **M-4′** is by 2.8 kcal mol^{-1} less stable than **M-4**. Deprotonation of intermediate **M-4** and isomer **M-4′** at carbon atom adjacent to the carbonyl group (Scheme 6) leads to formation of anion **M-5** and **M-5′**, respectively. In experimental conditions when the reaction is carried out in the presence of strong base, such as methoxide, deprotonation of intermediate compound (corresponding to model intermediate **M-4** and **M-4′**) immediately follows the first step and the five-membered ring intermediate cannot be isolated. However, in the presence of a weaker base, such as K_2CO_3 in acetonitrile or Et_3N, the reaction equilibrium is shifted toward the intermediate (corresponding to model intermediate **M-4** and **M-4′**) and resulting in the lower amount of required anion (corresponding to model anion **M-5** and **M-5′**). As an experimental confirmation compound **26** (corresponding to model intermediate **M-4′**) was isolated from the reaction mixture and the structure confirmed by X-ray crystallography (Fig. 5).

Following the E1cB elimination mechanism[37,38] we expected the direct subsequent elimination of the aryl amine group from model anion **M-5** and anion **M-5′**. However, this step is computationally not observed and elimination of aryl amine group occurs only after protonation of anion **M-5** and anion **M-5′** at the nitrogen atom of the amine group through intermediate **M-6** and **M-6′**, respectively. The elimination reaction then proceeds via the transition state **M-**

TS_2 leading to formation of product complex **M-7** (Fig. 8) in case of anion **M-5** and via the transition state **M-TS2′** giving product complex **M-7′** (Fig. 9) in case of anion **M5′**.

Fig. 8 shows cleavage of C–N bond of the leaving aryl amine group by lengthening of C–N bond from 1.601 Å in intermediate **M-6** to 4.402 Å in the final product complex **M-7** via transition state **M-TS_2**. Product complex **M-7** is stabilized by weak van der Waals interactions (as indicated by a very long C–N distance) and it readily dissociates giving final reaction products. The same process can be observed in the case of intermediate **M-6′** where the cleavage of C–N bond of the leaving aryl amine group occurs by lengthening of the bond from 1.603 Å in intermediate **M-6′** to 4.841 Å in the final product complex **M-7′** via transition state **M-TS_2′**, Fig. 9. The final products are isomeric forms which differ in the orientation of substituents around the newly formed double bond. The product **M-7′** is by 1.4 kcal mol^{-1} more stable then isomer **M-7** which can account for the fact that only one isomer can be detected in the reaction mixture. The overall schematic representation of the reaction mechanism is shown in Fig. 10.

2.3. Antiproliferative activity

Seven novel benzoylaminoimidazolones and six 2-aminoimidazolones were tested for their antiproliferative activity on HCT116 (colon carcinoma) and H460 (lung carcinoma) cell lines (Table 3). Some of the tested benzoylaminoimidazolones (**27**, **30**) were poorly soluble in DMSO and therefore, their results should be interpreted with caution. Most of the benzoylaminoimidazolones exhibited high activity with IC_{50} values in the micromolar range, which is comparable to IC_{50} values of cisplatin (IC_{50} 10 and 1 μM, respectively) and sorafenib (IC_{50} 3 and 3 μM, respectively), drugs commonly used in clinics. The most active compound was **17** with IC_{50} 1 and 2 μM in HCT116 and H460 cell line, respectively. Interestingly, benzoylaminoimidazolone **20** with cyclohexyl group at the 1-position of the imidazolone ring exhibited lower activity (IC_{50} 19 and 32 μM, respectively) than benzoylaminoimidazolones containing aryl substituents. On the other hand, 2-aminoimidazolones had on average 10-fold higher IC_{50} values. 2-Aminoimidazolone **24** was the most active with IC_{50} value 26 μM. Compounds **22**, **23** and

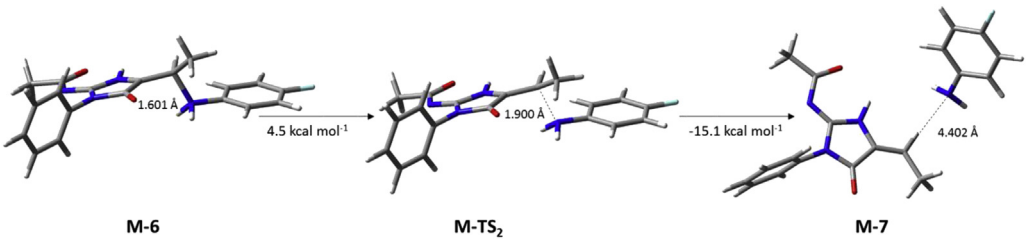


Fig. 8. Optimized structures of intermediate **M-6**, transition state **M-TS_2** and product complex **M-7** obtained at the SMD/B3LYP/6–31+G(d) level of theory with methanol as solvent.

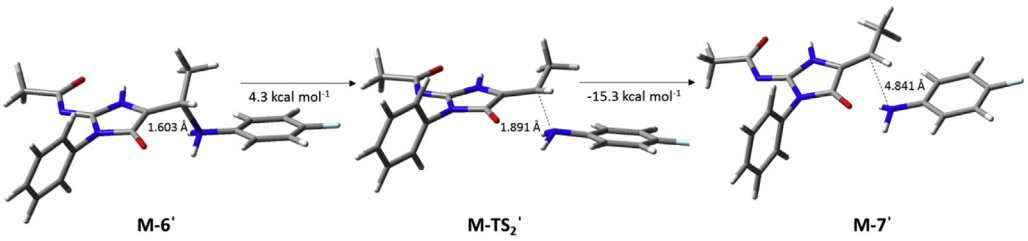


Fig. 9. Optimized structures of intermediate **M-6**, transition state **M-TS_2′** and product complex **M-7** obtained at the SMD/B3LYP/6–31+G(d) level of theory with methanol as solvent.

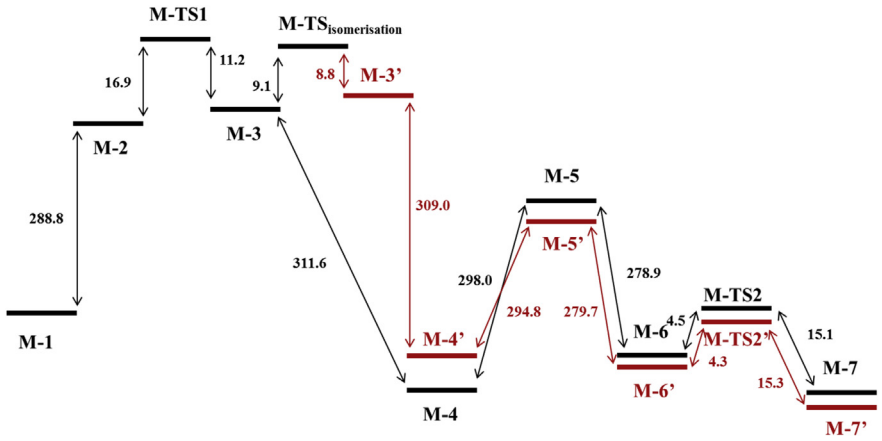


Fig. 10. Relative Gibbs free energy differences in kcal mol^{-1} obtained at the SMD/MP2/6–311++G(d,p)//B3LYP/6–31+G(d) level of theory with methanol as solvent.

Table 3
IC_{50} values (μM) of benzoylaminoimidazolones **16**–**20** and 2-aminoimidazolones **21**–**25**

Compound	IC_{50} (μM)	
	HCT116	H460
16	4±2.6	7±1.9
17	1±0.9	2±0.0
18	8±2.3	6±0.9
19	3±0.5	6±2.1
20	19±12.6	32±7.8
27	13±8.5[a]	17±8.8[a]
30	4±23.1[a]	32±3.1[a]
21	30±7.3	62±4.8
22	35±10.5	>100
23	57±16.0	>100
24	26±4.8	60±23.6
25	35±5.5	26±2.0
28	41±23.1	>100
Cisplatin	10±2.0	1±0.1
Sorafenib	3±0.8	3±0.8

IC_{50}: the concentration that causes 50% growth inhibition.
[a] The compounds precipitated in DMSO.

28 had IC_{50} values higher than 100 μM in H460 cells and were considered inactive. Therefore, it seems that benzoyl substituent at 2-amino group is necessary for high antiproliferative activity of tested aminoimidazolone compounds.

2.4. In vitro inhibition of β-secretase activity

Since 2-aminoimidazoles are known β-secretase inhibitors,[15–18] we tested in vitro β-secretase inhibitory activity of three novel 2-aminoimidazolones **21**, **22** and **23**. Chinese hamster ovary (CHO) cell line transiently transfected with amyloid precursor protein (APP) was used as a cell model. APP and sAPPβ levels were monitored after 24 h treatment by western blot analysis. While the known β-secretase inhibitor IV (C3) caused a complete loss in sAPPβ generation (due to β-secretase inhibition), tested compounds failed to show β-secretase inhibitory activity (Fig. S3, Supplementary data). Therefore, other 2-aminoimidazolones were not further tested. Benzoylaminoimidazolones were also not tested due to their high antiproliferative activity.

3. Conclusions

In conclusion, we have developed a facile and practical method for the synthesis of biologically active aminoimidazolones via the direct base-promoted transformation of β-lactam to imidazole ring. By using quantum chemical calculations, we showed that the reaction involves rearrangement of β-lactam guanidines followed by E1cB elimination at the 4-position of imidazole. New aminoimidazolones show strong antiproliferative activity, which is highly structure dependent.

4. Experimental section

4.1. General methods

Melting points were determined on a Reichert Thermovar 7905 apparatus and are uncorrected. The IR spectra were recorded on a PerkinElmer Spectrum RX I FTIR System spectrometer (KBr pellets technique) (PerkinElmer Instruments, Norwalk, CT, USA). The ^{1}H and ^{13}C NMR spectra (in $CDCl_3$ and DMSO-d_6 at RT) were recorded on a Bruker AV 300 and/or AV 600 spectrometer (Bruker BioSpin GmbH., Rheinstetten, Germany), δ is expressed in ppm relative to tetramethylsilane as an internal reference. Microanalysis was performed on a PE 2400 Series II CHNS/O Analyzer (PerkinElmer Instruments, Shelton, CT, USA). Samples for HRMS analysis were suspended in 5 μl of THAP/DAC matrix and 1 μl was spotted onto a MALDI plate. Mass spectra were obtained on a matrix-assisted laser desorption/ionization-time-of-flight MALDI-TOF/TOF mass spectrometer (4800 Plus MALDI-TOF/TOF Analyzer, Applied Biosystems, Foster City, CA, USA) equipped with Nd:YAG laser operating at 355 nm with firing rate 200 Hz in the positive ion reflector mode. 1600 shots per spectrum were taken covering mass range 100–1000 Da, focus mass 500 Da and delay time 100 ns.

CCDC 1045578–1045579 contain the supplementary crystallographic data for this paper, which can be obtained free of charge via www.ccdc.cam.uk/data_request/cif [Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, UK; Fax: +44 1223 336033; email: data_request@ccdc.cam.ac.uk]. Structure factor tables are available from the authors. Selected bond distances, angles, and details on hydrogen bond geometry are presented in Tables S2, S3, respectively (Supplementary data).

4.2. Synthesis of β-lactams 1a and 1b

*4.2.1. (3R,4R)-3-Amino-1-(4-fluorophenyl)-4-(4-methoxyphenyl)-2-azetidinone (**1a**).* Amino-β-lactam **1a** was synthesized according to the procedure described by Ojima et al.[34] and Poljak et al.[35] (1*R*,2*S*,5*R*)-(−)-menthol was used as chiral auxiliary. Crude product was purified by a silica gel column chromatography (EtOAc) and obtained as white crystals (1.09 g, 53%). $[\alpha]_D^{20}$ −20 (*c* 10 mg/mL EtOAc); mp 78–79 °C; FTIR (KBr) cm^{-1}: 2959, 2930, 1734, 1609, 1508, 1388, 1246, 1027, 835; ^{1}H NMR (300 MHz, $CDCl_3$): 7.22–7.27 (m, 4H, Ar–*H*), 6.88–6.95 (m, 4H, Ar–*H*), 4.59 (d, *J*=2.1 Hz, 1H, C3, β-lactam), 4.04 (d, *J*=2.1 Hz, 1H, C4, β-lactam), 3.80 (s, 3H, O*CH*$_3$), 1.76 (s, 2H, N*H*$_2$); ^{13}C NMR (75 MHz, $CDCl_3$): 168.0 (*C*]O, β-lactam); 160.0 (4-OCH_3–C_6H_4), 159.2 (d, *J*=243.9 Hz, 4-F–C_6H_4), 133.8 (d, *J*=2.7 Hz, 4-F–C_6H_4), 128.5 (4-OCH_3–C_6H_4), 127.3 (4-OCH_3–C_6H_4), 119.0 (d, *J*=7.8 Hz, 4-F–C_6H_4), 116.0 (d, *J*=22.8 Hz, 4-F–C_6H_4), 114.7 (4-OCH_3–C_6H_4), 70.2 (C3, β-lactam), 66.6 (C4, β-lactam), 55.5 (OCH_3); HRMS (MALDI-TOF/TOF) *m*/*z*: $[M+H]^+$ Anal. Calcd for $C_{16}H_{16}FN_2O_2$ 287.1190; Found 287.1199.

*4.2.2. (3S,4S)-3-Amino-1-(4-fluorophenyl)-4-(4-methoxyphenyl)-2-azetidinone (**1b**).* Amino-β-lactam **1b** was synthesized according to the procedure for **1a**. (1*S*,2*R*,5*S*)-(+)-menthol was used as chiral auxiliary. The product was obtained as white crystals (779 mg, 61%). $[\alpha]_D^{20}$ +20 (*c* 10 mg/mL EtOAc); mp 78–79 °C; FTIR (KBr) cm^{-1}: 2959, 2930, 1734, 1609, 1508, 1388, 1246, 1027, 835; ^{1}H NMR (300 MHz, $CDCl_3$): 7.22–7.27 (m, 4H, Ar–*H*), 6.88–6.95 (m, 4H, Ar–*H*), 4.59 (d, *J*=2.1 Hz, 1H, C3, β-lactam), 4.04 (d, *J*=2.1 Hz, 1H, C4, β-lactam), 3.80 (s, 3H, O*CH*$_3$), 1.76 (s, 2H, N*H*$_2$); ^{13}C NMR (75 MHz, $CDCl_3$): 168.0 (*C*]O, β-lactam), 160.0 (4-OCH_3–C_6H_4), 159.2 (d, *J*=243.9 Hz, 4-F–C_6H_4), 133.8 (d, *J*=2.7 Hz, 4-F–C_6H_4), 128.5 (4-OCH_3–C_6H_4), 127.3 (4-OCH_3–C_6H_4), 119.0 (d, *J*=7.8 Hz, 4-F–C_6H_4), 116.0 (d, *J*=22.8 Hz, 4-F–C_6H_4), 114.7 (4-OCH_3–C_6H_4), 70.2 (C3, β-lactam), 66.6 (C4, β-lactam), 55.5 (OCH_3); HRMS (MALDI-TOF/TOF) *m*/*z*: $[M+H]^+$ Anal. Calcd for $C_{16}H_{16}FN_2O_2$ 287.1190; Found 287.1199.

4.3. General procedure for the preparation of thioureas 2–8

Benzoyl isothiocyanate (1.0 equiv) was added to a solution of a corresponding amine (1.0 equiv) in acetonitrile (6 mL). The reaction proceeded overnight with stirring at room temperature, after, which the reaction mixture was evaporated to dryness and the crude product purified by silica gel column chromatography or recrystallization.

*4.3.1. N-(Phenylcarbamothioyl)benzamide (**2**).* Isolated as white crystals (489 mg, 73%) after silica gel column chromatography

(hexane/EtOAc 5:1). mp 140–141 °C; FTIR (KBr) cm^{-1}: 1670, 1606, 1560, 1540, 1361, 1256, 1146; ^1H NMR (300 MHz, $CDCl_3$): 12.57 (br s, 1H, N*H*), 9.08 (br s, 1H, N*H*), 7.87–7.90 (m, 2H, Ar–*H*), 7.72 (d, *J*=8.0 Hz, 2H, Ar–*H*), 7.62–7.67 (m, 1H, Ar–*H*), 7.53 (t, *J*=7.4 Hz, 2H, Ar–*H*), 7.42 (t, *J*=7.8 Hz, 2H, Ar–*H*), 7.28 (t, *J*=7.7 Hz, 1H, Ar–*H*); ^{13}C NMR (75 MHz, $CDCl_3$): 178.5 (*C*=S), 167.1 (*C*=O), 137.8 (C_6H_5), 133.9 (C_6H_5), 131.8 (C_6H_5), 129.4 (C_6H_5), 129.0 (C_6H_5), 127.6 (C_6H_5), 127.0 (C_6H_5), 124.2 (C_6H_5); Anal. Calcd for $C_{14}H_{12}N_2OS$: C, 65.60; H, 4.72; N, 10.93. Found: C, 65.23; H, 4.90; N, 10.92.

*4.3.2. N-(4-Fluorophenylcarbamothioyl)benzamide (**3**).* Isolated as yellow crystals (500 mg, 70%) after silica gel column chromatography (hexane/EtOAc 5:1). mp 120–121 °C; FTIR (KBr) cm^{-1}: 3225, 3133, 3035, 1670, 1508, 1329, 1260, 1153, 838, 805; ^1H NMR (300 MHz, $CDCl_3$): 12.50 (br s, 1H, N*H*), 9.07 (br s, 1H, N*H*), 7.87–7.90 (m, 2H, Ar–*H*), 7.64–7.68 (m, 3H, Ar–*H*), 7.55 (t, *J*=7.5 Hz, 2H, Ar–*H*), 7.11 (t, *J*=8.6 Hz, 2H, Ar–*H*); ^{13}C NMR (75 MHz, $CDCl_3$): 178.9 (*C*]S), 167.1 (*C*]O), 161.0 (d, *J*=247.1 Hz, 4-F–C_6H_4), 133.8 (C_6H_5), 133.7 (d, *J*=3.0 Hz, 4-F–C_6H_4), 131.6 (C_6H_5), 129.2 (C_6H_5), 127.6 (C_6H_5), 126.3 (d, *J*=8.4 Hz, 4-F–C_6H_4), 115.8 (d, *J*=22.9 Hz, 4-F–C_6H_4); Anal. Calcd for $C_{14}H_{11}FN_2OS$: C, 61.30; H, 4.04; N, 10.21. Found: C, 61.12; H, 4.42; N, 10.22.

*4.3.3. N-(4-Methoxyphenylcarbamothioyl)benzamide (**4**).* Isolated as white crystals (452 mg, 60%) after silica gel column chromatography (hexane/acetone 2:1). mp 148–149 °C; FTIR (KBr) cm^{-1}: 3229, 3032, 1668, 1535, 1510, 1338, 1270, 1248, 1154, 1027, 834; ^1H NMR (300 MHz, $CDCl_3$): 12.39 (br s, 1H, N*H*), 9.07 (br s, 1H, N*H*), 7.88–7.89 (m, 2H, Ar–*H*), 7.51–7.67 (m, 5H, Ar–*H*), 6.93 (d, *J*=8.8 Hz, 2H, Ar–*H*), 3.82 (s, 3H, O*CH*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): 178.7 (*C*]S), 167.0 (*C*]O), 158.4 (4-OCH_3–C_6H_4), 133.8 (C_6H_5), 131.9 (C_6H_5), 130.7 (4-OCH_3–C_6H_4), 129.4 (C_6H_5), 127.6 (C_6H_5), 125.9 (4-O*CH*$_3$–C_6H_4), 114.3 (4-OCH_3–C_6H_4), 55.6 (O*CH*$_3$); Anal. Calcd for $C_{15}H_{14}N_2O_2S$: C, 62.92; H, 4.93; N, 9.78. Found: C, 62.71; H, 5.23; N, 9.99.

*4.3.4. N-(4-Methylphenylcarbamothioyl)benzamide (**5**).* Isolated as white crystals (632 mg, 90%) after silica gel column chromatography (hexane/EtOAc/CH_2Cl_2 5:1:1). mp 146–147 °C; FTIR (KBr) cm^{-1}: 3467, 1670, 1599, 1508, 1354, 1255, 1147, 816; ^1H NMR (300 MHz, $CDCl_3$): 12.46 (br s, 1H, N*H*), 9.05 (br s, 1H, N*H*), 7.87–7.90 (m, 2H, Ar–*H*), 7.62–7.67 (m, 1H, Ar–*H*), 7.51–7.58 (m, 4H, Ar–*H*), 7.20–7.25 (m, 2H, Ar–*H*), 2.36 (s, 3H, C*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): 178.6 (*C*]S), 167.0 (*C*]O), 137.0 (4-CH_3–C_6H_4), 135.2 (4-CH_3–C_6H_4), 133.9 (C_6H_5), 131.9 (C_6H_5), 129.7 (4-CH_3–C_6H_4), 129.4 (C_6H_5), 127.6 (C_6H_5), 124.3 (4-CH_3–C_6H_4), 21.2 (*CH*$_3$); Anal. Calcd for $C_{15}H_{14}N_2OS$: C, 66.64; H, 5.22; N, 10.36. Found: C, 66.58; H, 5.62; N, 10.53.

*4.3.5. N-(Cyclohexylcarbamothioyl)benzamide (**6**).* Isolated as white crystals (579 mg, 85%) after silica gel column chromatography (hexane/EtOAc 5:1). mp 61–62 °C; FTIR (KBr) cm^{-1}: 3170, 2927, 2853, 1672, 1544, 1341, 1262, 1171; ^1H NMR (300 MHz, $CDCl_3$): 10.71 (br s, 1H, N*H*), 8.91 (br s, 1H, N*H*), 7.81–7.84 (m, 2H, Ar–*H*), 7.59–7.64 (m, 1H, Ar–*H*), 7.48–7.53 (m, 2H, Ar–*H*), 4.25–4.34 (m, 1H, cyclohexyl), 2.03–2.11 (m, 2H, cyclohexyl), 1.73–1.77 (m, 2H, cyclohexyl), 1.58–1.66 (m, 1H, cyclohexyl), 1.23–1.52 (m, 5H, cyclohexyl); ^{13}C NMR (75 MHz, $CDCl_3$): 178.4 (*C*]S), 166.9 (*C*]O), 133.6 (C_6H_5), 132.0 (C_6H_5), 129.3 (C_6H_5), 127.5 (C_6H_5), 54.5 (cyclohexyl), 31.7 (cyclohexyl), 25.6 (cyclohexyl), 24.5 (cyclohexyl); HRMS (MALDI-TOF/TOF) *m*/*z*: $[M+H]^+$ calcd for $C_{14}H_{19}N_2OS$ 263.1212; Found 263.1221.

*4.3.6. N-(4-Cyanophenylcarbamothioyl)benzamide (**7**).* Isolated as light yellow crystals (582 mg, 79%) after silica gel column chromatography (hexane/EtOAc 5:1). mp 166–167 °C; FTIR (KBr) cm^{-1}: 3481, 3236, 1664, 1598, 1522, 1341, 1266, 1165, 836; ^1H NMR (600 MHz, $CDCl_3$): 12.96 (br s, 1H, N*H*), 9.09 (br s, 1H, N*H*), 7.99 (d, *J*=8.7 Hz, 2H, Ar–*H*), 7.90 (d, *J*=7.3 Hz, 2H, Ar–*H*), 7.67–7.71 (m, 3H, Ar–*H*), 7.57 (t, *J*=7.8 Hz, 2H, Ar–*H*); ^{13}C NMR (150 MHz, $CDCl_3$): 178.3 (*C*]S), 167.3 (*C*]O), 141.7 (4-CN–C_6H_4), 134.3 (4-CN–C_6H_4), 133.1 (C_6H_5), 131.4 (C_6H_5), 129.5 (C_6H_5), 127.7 (C_6H_5), 123.7 (4-CN–C_6H_4), 118.6 (4-CN–C_6H_4), 109.9 (*C*≡N); Anal. Calcd for $C_{15}H_{11}N_3OS$: C, 64.04; H, 3.94; N, 14.94. Found: C, 64.16; H, 3.84; N, 15.01.

*4.3.7. N-(4-Nitrophenylcarbamothioyl)benzamide (**8**).* Isolated as yellow crystals (691 mg, 88%) after recrystallization in acetonitrile. mp 175–176 °C; FTIR (KBr) cm^{-1}: 3249, 1667, 1577, 1515, 1316, 1267, 1166, 850; ^1H NMR (300 MHz, $CDCl_3$): 13.07 (br s, 1H, N*H*), 9.12 (br s, 1H, N*H*), 8.29 (d, *J*=9.1 Hz, 2H, Ar–*H*), 8.06 (d, *J*=9.1 Hz, 2H, Ar–*H*), 7.91 (d, *J*=7.3 Hz, 2H, Ar–*H*), 7.54–7.71 (m, 3H, Ar–*H*); ^{13}C NMR (75 MHz, $CDCl_3$): 178.3 (*C*]S), 167.3 (*C*]O), 145.4 (4-NO_2–C_6H_4), 143.4 (4-NO_2–C_6H_4), 134.3 (C_6H_5), 131.4 (C_6H_5), 129.5 (C_6H_5), 127.7 (C_6H_5), 124.8 (4-NO_2–C_6H_4), 123.3 (4-NO_2–C_6H_4); Anal. Calcd for $C_{14}H_{11}N_3O_3S$: C, 55.80; H, 3.68; N, 13.95. Found: C, 55.83; H, 3.84; N, 13.94.

4.4. General procedure for the preparation of guanidines 9–13

Guanidines **9**–**13** were synthesized according to the procedure described by Cunha et al.[36] To a solution of corresponding thiourea **2**–**6** (1.0 equiv) in DMF (5 mL) β-lactam **1a** or **1b** (1.0 equiv) and Et_3N (2.0 equiv) was added, after which the reaction mixture was cooled to 0 °C and $HgCl_2$ (1.0 equiv) added. The reaction mixture was stirred vigorously overnight, filtered and the filtrate evaporated to dryness. Deionized water was added to the filtrate and the resulting mixture extracted with ethyl acetate (3×20 mL). Combined organic layers were dried on Na_2SO_4, filtered and evaporated. Crude products **9**–**13** were purified by either silica gel column chromatography (hexane/EtOAc 2:1) or recrystallization.

*4.4.1. N-((((3R,4R)-1-(4-Fluorophenyl)-4-(4-methoxyphenyl)-2-oxoazetidin-3-yl)amino)(phenylamino)methylene)benzamide (**9a**).* Isolated as white crystals (183 mg, 60%) after recrystallization from diethyl ether. $[\alpha]_D^{20}$ +21 (*c* 10 mg/mL EtOAc); mp 165–166 °C; FTIR (KBr) cm^{-1}: 3468, 3271, 1742, 1601, 1570, 1508, 1354, 1229, 1024, 830; ^1H NMR (300 MHz, $CDCl_3$): 12.22 (br s, 1H, N*H*), 7.77 (d, *J*=7.4 Hz, 2H, Ar–*H*), 7.40–7.46 (m, 2H, Ar–*H*), 7.26–7.32 (m, 8H, Ar–*H*), 6.87–6.98 (m, 6H, Ar–*H*), 5.54 (br s, 1H, N*H*), 5.25 (d, *J*=2.1 Hz, 1H, C4, β-lactam), 4.65 (dd, *J*=6.7 Hz, 2.2 Hz, 1H, C3, β-lactam), 3.80 (s, 3H, O*CH*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): 178.4 (N-(*C*]O)C_6H_5), 164.2 (*C*]O, β-lactam), 160.3 (4-OCH_3–C_6H_4), 159.3 (d, *J*=243.9 Hz, 4-F–C_6H_4), 158.0 (*C*]N, guanidine), 137.6 (C_6H_5), 135.5 (C_6H_5), 133.8 (d, *J*=2.8 Hz, 4-F–C_6H_4), 131.4 (C_6H_5), 130.4 (C_6H_5), 129.3 (C_6H_5), 128.3 (4-OCH_3–C_6H_4), 127.9 (4-OCH_3–C_6H_4), 127.72 (C_6H_5), 127.65 (C_6H_5), 125.9 (C_6H_5), 119.2 (d, *J*=7.8 Hz, 4-F–C_6H_4), 116.1 (d, *J*=22.6 Hz, 4-F–C_6H_4), 114.9 (4-OCH_3–C_6H_4), 67.8 (C3, β-lactam), 63.4 (C4, β-lactam), 55.5 (OCH_3); Anal. Calcd for $C_{30}H_{25}FN_4O_3$: C, 70.85; H, 4.95; N, 11.02. Found: C, 70.51; H, 5.15; N, 10.57.

*4.4.2. N-((((3S,4S)-1-(4-Fluorophenyl)-4-(4-methoxyphenyl)-2-oxoazetidin-3-yl)amino)(phenylamino)methylene)benzamide (**9b**).* Isolated as white crystals (115 mg, 38%) after recrystallization from diethyl ether. $[\alpha]_D^{20}$ −21 (*c* 10 mg/mL EtOAc); mp 165–166 °C; FTIR (KBr) cm^{-1}: 3468, 3271, 1742, 1601, 1570, 1508, 1354, 1229, 1024, 830; ^1H NMR (300 MHz, $CDCl_3$): 12.22 (br s, 1H, N*H*), 7.77 (d, *J*=7.4 Hz, 2H, Ar–*H*), 7.40–7.46 (m, 2H, Ar–*H*), 7.26–7.32 (m, 8H, Ar–*H*), 6.87–6.98 (m, 6H, Ar–*H*), 5.54 (br s, 1H, N*H*), 5.25 (d, *J*=2.1 Hz, 1H, C4, β-lactam), 4.65 (dd, *J*=6.7 Hz, 2.2 Hz, 1H, C3, β-lactam), 3.80 (s, 3H, O*CH*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): 178.4 (N-(*C*]O)C_6H_5), 164.2 (*C*]O, β-lactam), 160.3 (4-OCH_3–C_6H_4), 159.3 (d, *J*=243.9 Hz, 4-F–C_6H_4), 158.0 (*C*]N, guanidine), 137.6 (C_6H_5), 135.5 (C_6H_5),

133.8 (d, J=2.8 Hz, 4-F–C_6H_4), 131.4 (C_6H_5), 130.4 (C_6H_5), 129.3 (C_6H_5), 128.3 (4-OCH_3–C_6H_4), 127.9 (4-OCH_3–C_6H_4), 127.72 (C_6H_5), 127.65 (C_6H_5), 125.9 (C_6H_5), 119.2 (d, J=7.8 Hz, 4-F–C_6H_4), 116.1 (d, J=22.6 Hz, 4-F–C_6H_4), 114.9 (4-OCH_3–C_6H_4), 67.8 (C3, β-lactam), 63.4 (C4, β-lactam), 55.5 (OCH_3); Anal. Calcd for $C_{30}H_{25}FN_4O_3$: C, 70.85; H, 4.95; N, 11.02. Found: C, 70.51; H, 5.15; N, 10.57.

*4.4.3. N-((((3R,4R)-1-(4-Fluorophenyl)-4-(4-methoxyphenyl)-2-oxoazetidin-3-yl)amino) (4-fluorophenylamino)methylene)benzamide (**10**).* Isolated as white crystals (284 mg, 77%) after silica gel column chromatography. $[\alpha]_D^{20}$ +40 (*c* 10 mg/mL EtOAc); mp 156–157 °C; FTIR (KBr) cm^{-1}: 3468, 1740, 1560, 1508, 1355, 1227, 830; ^1H NMR (300 MHz, $CDCl_3$): 12.14 (br s, 1H, N*H*), 7.76 (d, J=7.4 Hz, 2H, Ar–*H*), 7.25–7.32 (m, 7H, Ar–*H*), 7.12 (t, J=8.5 Hz; 2H, Ar–*H*), 6.88–6.98 (m, 6H, Ar–*H*), 5.40 (br s, 1H, N*H*), 5.24 (d, J=2.2 Hz, 1H, C4, β-lactam), 4.65 (dd, J=6.7 Hz, 2.3 Hz, 1H, C3, β-lactam), 3.80 (s, 3H, O*CH*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): 178.4 (N-(*C*]O)C_6H_5), 164.3 (*C*]O, β-lactam), 163.5 (d, J=254.9 Hz, 4-F–C_6H_4), 160.3 (4-OCH_3–C_6H_4), 159.3 (d, J=244.5 Hz, 4-F–C_6H_4), 158.3 (*C*]N, guanidine), 137.4 (C_6H_5), 133.7 (4-F–C_6H_4), 131.5 (C_6H_5), 131.3 (4-F–C_6H_4), 129.3 (C_6H_5), 128.3 (d, J=7.1 Hz, 4-F–C_6H_4), 128.2 (4-OCH_3–C_6H_4), 127.8 (4-OCH_3–C_6H_4), 127.7 (C_6H_5), 119.1 (d, J=7.9 Hz, 4-F–C_6H_4), 117.3 (d, J=23.0 Hz, 4-F–C_6H_4), 116.1 (d, J=22.6 Hz, 4-F–C_6H_4), 114.9 (4-OCH_3–C_6H_4), 67.7 (C3, β-lactam), 63.5 (C4, β-lactam), 55.5 (OCH_3); Anal. Calcd for $C_{30}H_{24}F_2N_4O_3$: C, 68.43; H, 4.59; N, 10.64. Found: C, 68.25; H, 4.68; N, 10.86.

*4.4.4. N-((((3R,4R)-1-(4-Fluorophenyl)-4-(4-methoxyphenyl)-2-oxoazetidin-3-yl)amino) (4-methoxyphenylamino)methylene)benzamide (**11**).* Isolated as white crystals (294 mg, 78%) after silica gel column chromatography. $[\alpha]_D^{20}$ +34 (*c* 10 mg/mL EtOAc); mp 160–161 °C; FTIR (KBr) cm^{-1}: 3468, 3265, 1742, 1600, 1560, 1508, 1357, 1248, 1150, 1034, 831; ^1H NMR (300 MHz, $CDCl_3$): 11.96 (br s, 1H, N*H*), 7.73 (d, J=7.5 Hz, 2H, Ar–*H*), 7.20–7.31 (m, 7H, Ar–*H*), 6.86–6.98 (m, 8H, Ar–*H*), 5.70 (d, J=6.0 Hz, 1H, N*H*), 5.19 (d, J=1.9 Hz, 1H, C4, β-lactam), 4.65 (dd, J=7.0 Hz, 1.8 Hz, 1H, C3, β-lactam), 3.80 (s, 3H, O*CH*$_3$), 3.72 (s, 3H, O*CH*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): 178.1 (N-(*C*]O)C_6H_5), 164.5 (*C*]O, β-lactam), 160.2 (4-OCH_3–C_6H_4), 159.3 (d, J=244.1 Hz, 4-F–C_6H_4), 159.1 (4-OCH_3–C_6H_4), 158.7 (*C*]N, guanidine), 137.7 (C_6H_5), 133.7 (d, J=2.7 Hz, 4-F–C_6H_4), 131.3 (C_6H_5), 129.2 (C_6H_5), 128.3 (4-OCH_3–C_6H_4), 127.9 (C_6H_5), 127.8 (4-OCH_3–C_6H_4), 127.7 (4-OCH_3–C_6H_4), 127.6 (4-OCH_3–C_6H_4), 119.0 (d, J=7.9 Hz, 4-F–C_6H_4), 116.0 (d, J=22.7 Hz, 4-F–C_6H_4), 115.5 (4-OCH_3–C_6H_4), 114.8 (4-OCH_3–C_6H_4), 67.7 (C3, β-lactam), 63.6 (C4, β-lactam), 55.6 (OCH_3), 55.4 (OCH_3); Anal. Calcd for $C_{31}H_{27}FN_4O_4$: C, 69.13; H, 5.05; N, 10.40. Found: C, 68.78; H, 5.30; N, 10.30.

*4.4.5. N-((((3R,4R)-1-(4-Fluorophenyl)-4-(4-methoxyphenyl)-2-oxoazetidin-3-yl)amino) (4-methylphenylamino)methylene)benzamide (**12**).* Isolated as white crystals (284 mg, 78%) after silica gel column chromatography. $[\alpha]_D^{20}$ +29 (*c* 10 mg/mL EtOAc); mp 121–122 °C; FTIR (KBr) cm^{-1}: 3466, 3266, 1741, 1600, 1570, 1509, 1355, 1249, 1226, 1033, 831; ^1H NMR (300 MHz, $CDCl_3$): 12.09 (br s, 1H, N*H*), 7.76 (d, J=7.1 Hz, 2H, Ar–*H*), 7.17–7.31 (m, 9H, Ar–*H*), 6.86–6.98 (m, 6H, Ar–*H*), 5.50 (d, J=6.0 Hz, 1H, N*H*), 5.24 (d, J=2.2 Hz, 1H, C4, β-lactam), 4.62 (dd, J=6.7 Hz, 2.2 Hz, 1H, C3, β-lactam), 3.80 (s, 3H, O*CH*$_3$), 2.34 (s, 3H, C*H*$_3$); ^{13}C NMR (150 MHz, $CDCl_3$): 178.3 (N-(*C*]O)C_6H_5), 164.3 (*C*]O, β-lactam), 160.3 (4-OCH_3–C_6H_4), 159.3 (d, J=244.2 Hz, 4-F–C_6H_4), 158.3 (*C*]N, guanidine), 137.9 (4-CH_3–C_6H_4), 137.6 (C_6H_5), 133.9 (d, J=1.6 Hz, 4-F–C_6H_4), 132.7 (4-CH_3–C_6H_4), 131.4 (C_6H_5), 131.0 (C_6H_5), 129.3 (4-CH_3–C_6H_4), 128.3 (4-OCH_3–C_6H_4), 127.9 (4-OCH_3–C_6H_4), 127.6 (C_6H_5), 126.0 (4-CH_3–C_6H_4), 119.2 (d, J=7.8 Hz, 4-F–C_6H_4), 116.1 (d, J=22.6 Hz, 4-F–C_6H_4), 114.9 (4-OCH_3–C_6H_4), 67.9 (C3, β-lactam), 63.5 (C4, β-lactam), 55.5 (OCH_3), 21.2 (CH_3); Anal. Calcd for $C_{31}H_{27}FN_4O_3$: C, 71.25; H, 5.21; N, 10.72. Found: C, 70.97; H, 5.53; N, 10.56.

*4.4.6. N-((((3R,4R)-1-(4-Fluorophenyl)-4-(4-methoxyphenyl)-2-oxoazetidin-3-yl)amino) (cyclohexylamino)methylene)benzamide (**13**).* Isolated as white crystals (310 mg, 87%) after silica gel column chromatography. $[\alpha]_D^{20}$ +25 (*c* 10 mg/mL EtOAc); mp 122–123 °C; FTIR (KBr) cm^{-1}: 3315, 2931, 2852, 1733, 1609, 1511, 1393, 1357, 1229, 1030, 886, 831; ^1H NMR (300 MHz, $CDCl_3$): 10.67 (br s, 1H, N*H*), 7.66 (br s, 2H, Ar–*H*), 7.22–7.32 (m, 5H, Ar–*H*), 6.89–6.95 (m, 6H, Ar–*H*), 5.71 (br s, 1H, N*H*), 5.18 (s, 1H, C4, β-lactam), 4.86 (br s, 1H, C3, β-lactam), 3.81 (s, 3H, O*CH*$_3$), 3.35 (br s, 1H, cyclohexyl), 1.97–2.03 (m, 2H, cyclohexyl), 1.73–1.75 (m, 2H, cyclohexyl), 1.21–1.46 (m, 6H, cyclohexyl); ^{13}C NMR (75 MHz, $CDCl_3$): 177.6 (N-(*C*]O)C_6H_5), 162.7 (*C*]O, β-lactam), 160.3 (4-OCH_3–C_6H_4), 159.4 (d, J=244.5 Hz, 4-F–C_6H_4), 158.4 (*C*]N, guanidine), 138.2 (C_6H_5), 133.5 (d, J=1.8 Hz, 4-F–C_6H_4), 130.9 (C_6H_5), 129.0 (C_6H_5), 127.8 (4-OCH_3–C_6H_4), 127.5 (C_6H_5), 126.2 (4-OCH_3–C_6H_4), 119.1 (d, J=7.9 Hz, 4-F–C_6H_4), 116.0 (d, J=22.8 Hz, 4-F–C_6H_4), 114.9 (4-OCH_3–C_6H_4), 67.7 (C3, β-lactam), 64.3 (C4, β-lactam), 55.5 (OCH_3), 50.5 (cyclohexyl), 33.1 (cyclohexyl), 32.9 (cyclohexyl), 25.4 (cyclohexyl), 24.46 (cyclohexyl), 24.39 (cyclohexyl); HRMS (MALDI-TOF/TOF) *m/z*: $[M+H]^+$ calcd for $C_{30}H_{32}FN_4O_3$ 515.2452; Found 515.2456.

4.5. General procedure for the preparation of benzoylaminoimidazolones 16–20 and 2-aminoimidazolones 21–25

To a solution of corresponding guanidine **9**–**13** (1.0 equiv) in methanol (14 mL) K_2CO_3 (2.0 equiv) was added. The reaction mixture was stirred overnight at room temperature, after which it was evaporated to dryness. Distilled water was added and the resulting mixture extracted with dichloromethane (3×30 mL). Combined organic layers were dried on Na_2SO_4, filtered and evaporated. Crude products **16**–**20** and **21**–**25** were purified by silica gel column chromatography. Dichloromethane/hexane/ethyl acetate 3:3:1 was used to elute benzoylaminoimidazolones **16**–**20**, after which the eluent was changed to acetone to elute 2-aminoimidazolones **21**–**25**.

*4.5.1. (E)-N-((Z)-4-((4-Methoxyphenyl)methylidene)-5-oxo-1-phenyl-1,3-dihydroimidazol-2-ylidene)benzamide (**16**).* Synthesized from either guanidine **9a** or **9b**. Isolated as yellow to green powder (86 mg, 82%) for both **9a** and **9b**. mp 187–188 °C; FTIR (KBr) cm^{-1}: 3447, 1743, 1633, 1585, 1439, 1399, 1334, 1185, 1014, 817; ^1H NMR (600 MHz, $CDCl_3$): 11.92 (br s, 1H, N*H*), 8.14 (d, J=8.0 Hz, 2H, Ar–*H*), 7.45–7.58 (m, 8H, Ar–*H*), 7.38 (t, J=7.7 Hz, 2H, Ar–*H*), 7.05 (d, J=8.4 Hz, 2H, Ar–*H*), 6.95 (s, 1H, C*H*), 3.87 (s, 3H, O*CH*$_3$); ^{13}C NMR (150 MHz, $CDCl_3$): 179.5 (N-(*C*]O)C_6H_5), 162.8 (*C*]O, imidazolone), 161.2 (4-OCH_3–C_6H_4), 156.7 (*C*]N), 136.7 (C_6H_5), 132.7 (C_6H_5), 131.8 (C_6H_5), 131.3 (C_6H_5), 130.0 (C_6H_5), 129.1 (C_6H_5), 128.6 (C_6H_5), 128.3 (4-OCH_3–C_6H_4), 127.3 (C_6H_5), 125.5 (*C*]CH), 123.2 (4-OCH_3–C_6H_4), 115.9 (C]CH), 115.4 (4-OCH_3–C_6H_4), 55.6 (OCH_3); Anal. Calcd for $C_{24}H_{19}N_3O_3$: C, 72.53; H, 4.82; N, 10.57. Found: C, 72.49; H, 5.27; N, 10.81.

*4.5.2. (E)-N-((Z)-4-((4-Methoxyphenyl)methylidene)-5-oxo-1-(4-fluorophenyl)-1,3-dihydroimidazol-2-ylidene)benzamide (**17**).* Isolated as yellow to green powder (77 mg, 83%). mp 225–226 °C; FTIR (KBr) cm^{-1}: 3458, 1742, 1636, 1587, 1514, 1331, 1241, 1183, 1149, 1012, 815; ^1H NMR (300 MHz, $CDCl_3$): 11.91 (br s, 1H, N*H*), 8.11–8.15 (m, 2H, Ar–*H*), 7.48–7.58 (m, 5H, Ar–*H*), 7.40 (t, J=7.5 Hz, 2H, Ar–*H*), 7.24 (t, J=8.5 Hz, 2H, Ar–*H*), 7.06 (d, J=8.7 Hz, 2H, Ar–*H*), 6.97 (s, 1H, C*H*), 3.89 (s, 3H, O*CH*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): 179.3 (N-(*C*]O)C_6H_5), 162.7 (*C*]O, imidazolone), 162.2 (d, J=249.0 Hz, 4-F–C_6H_4), 161.3 (4-OCH_3–C_6H_4), 156.6 (*C*]N), 136.6

(C_6H_5), 132.8 (C_6H_5), 131.4 (C_6H_5), 129.9 (C_6H_5), 129.2 (d, J=8.6 Hz, 4-F–C_6H_4), 128.4 (4-OCH_3–C_6H_4), 127.7 (d, J=3.3 Hz, 4-F–C_6H_4), 125.5 (*C*]CH), 123.0 (4-OCH_3–C_6H_4), 116.2 (C]*C*H), 116.1 (d, J=23.1 Hz, 4-F–C_6H_4), 115.4 (4-OCH_3–C_6H_4), 55.7 (OCH_3); Anal. Calcd for $C_{24}H_{18}FN_3O_3$: C, 69.39; H, 4.37; N, 10.12. Found: C, 69.48; H, 4.47; N, 10.41.

*4.5.3. (E)-N-((Z)-4-((4-Methoxyphenyl)methylidene)-5-oxo-1-(4-methoxyphenyl)-1,3-dihydroimidazol-2-ylidene)benzamide (**18**).* Isolated as yellow to green powder (98 mg, 92%). mp 248–249 °C; FTIR (KBr) cm^{-1}: 3429, 1741, 1661, 1616, 1580, 1560, 1512, 1449, 1336, 1306, 1259, 1165, 1018, 829; ^1H NMR (600 MHz, $CDCl_3$): 11.87 (br s, 1H, N*H*), 8.14 (d, J=7.7 Hz, 2H, Ar–*H*), 7.42–7.52 (m, 5H, Ar–*H*), 7.38 (t, J=7.5 Hz, 2H, Ar–*H*), 7.04–7.06 (m, 4H, Ar–*H*), 6.95 (s, 1H, C*H*), 3.89 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3); ^{13}C NMR (150 MHz, $CDCl_3$): 179.5 (N-(*C*]O)C_6H_5), 163.0 (*C*]O, imidazolone), 161.2 (4-OCH_3–C_6H_4), 159.6 (4-OCH_3–C_6H_4), 157.0 (*C*]N), 136.8 (C_6H_5), 132.7 (C_6H_5), 131.3 (C_6H_5), 130.0 (C_6H_5), 128.5 (4-OCH_3–C_6H_4), 128.3 (4-OCH_3–C_6H_4), 125.6 (*C*]CH), 124.5 (4-OCH_3–C_6H_4), 123.3 (4-OCH_3–C_6H_4), 115.8 (C]*C*H), 115.4 (4-OCH_3–C_6H_4), 114.4 (4-OCH_3–C_6H_4), 55.69 (OCH_3), 55.62 (OCH_3); Anal. Calcd for $C_{25}H_{21}N_3O_4$: C, 70.25; H, 4.95; N, 9.83. Found: C, 70.22; H, 5.22; N, 9.54.

*4.5.4. (E)-N-((Z)-4-((4-Methoxyphenyl)methylidene)-5-oxo-1-(4-methylphenyl)-1,3-dihydroimidazol-2-ylidene)benzamide (**19**).* Isolated as yellow to green powder (79 mg, 69%). mp 245–246 °C; FTIR (KBr) cm^{-1}: 3462, 1740, 1663, 1618, 1579, 1514, 1440, 1393, 1330, 1259, 1236, 1166, 1077, 1016, 821; ^1H NMR (300 MHz, $CDCl_3$): 11.90 (br s, 1H, N*H*), 8.13–8.16 (m, 2H, Ar–*H*), 7.33–7.54 (m, 9H, Ar–*H*), 7.05 (d, J=8.8 Hz, 2H, Ar–*H*), 6.95 (s, 1H, C*H*), 3.88 (s, 3H, OCH_3), 2.45 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): 179.6 (N-(*C*]O)C_6H_5), 162.9 (*C*]O, imidazolone), 161.2 (4-OCH_3–C_6H_4), 156.9 (*C*]N), 138.6 (4-CH_3–C_6H_4), 136.8 (C_6H_5), 132.7 (C_6H_5), 131.3 (C_6H_5), 130.0 (C_6H_5), 129.7 (4-CH_3–C_6H_4), 129.1 (4-CH_3–C_6H_4), 128.3 (4-OCH_3–C_6H_4), 127.1 (4-CH_3–C_6H_4), 125.6 (*C*]CH), 123.3 (4-OCH_3–C_6H_4), 115.8 (C]*C*H), 115.4 (4-OCH_3–C_6H_4), 55.6 (OCH_3), 21.4 (CH_3); Anal. Calcd for $C_{25}H_{21}N_3O_3$: C, 72.98; H, 5.14; N, 10.21. Found: C, 72.79; H, 5.39; N 10.38.

*4.5.5. (E)-N-((Z)-4-((4-Methoxyphenyl)methylidene)-5-oxo-1-cyclohexyl-1,3-dihydroimidazol-2-ylidene)benzamide (**20**).* Isolated as yellow to green powder (81 mg, 66%). mp 143–144 °C; FTIR (KBr) cm^{-1}: 3461, 2939, 2852, 1726, 1664, 1616, 1582, 1565, 1512, 1444, 1312, 1297, 1276, 1261, 1179, 1134, 1043, 1019, 890, 830; ^1H NMR (300 MHz, $CDCl_3$): 11.77 (br s, 1H, N*H*), 8.27–8.31 (m, 2H, Ar–*H*), 7.45–7.57 (m, 5H, Ar–*H*), 7.02 (d, J=8.8 Hz, 2H, Ar–*H*), 6.81 (s, 1H, C*H*), 4.33–4.42 (m, 1H, cyclohexyl), 3.86 (s, 3H, OCH_3), 2.38–2.50 (m, 2H, cyclohexyl), 1.74–1.95 (m, 5H, cyclohexyl), 1.25–1.50 (m, 3H, cyclohexyl); ^{13}C NMR (75 MHz, $CDCl_3$): 179.2 (N-(*C*]O)C_6H_5), 163.6 (*C*]O, imidazolone), 160.9 (4-OCH_3–C_6H_4), 157.4 (*C*]N), 137.1 (C_6H_5), 132.6 (C_6H_5), 131.2 (C_6H_5), 129.8 (C_6H_5), 128.4 (4-OCH_3–C_6H_4), 125.8 (*C*]CH), 123.7 (4-OCH_3–C_6H_4), 115.3 (4-OCH_3–C_6H_4), 114.8 (C]*C*H), 55.6 (OCH_3), 52.8 (cyclohexyl), 29.6 (cyclohexyl), 26.2 (cyclohexyl), 25.5 (cyclohexyl); Anal. Calcd for $C_{24}H_{25}N_3O_3$: C, 71.44; H, 6.25; N, 10.41. Found: C, 71.22; H, 6.48; N, 10.53.

*4.5.6. (Z)-4-[(4-Methoxyphenyl)methylidene]-2-amino-1-phenyl-1H-imidazol-5(4H)-one (**21**).* Synthesized from either guanidine **9a** or **9b**. Isolated as yellow crystals (13 mg, 17%) for both **9a** and **9b**. mp 192–193 °C; FTIR (KBr) cm^{-1}: 3462, 2956, 1725, 1685, 1633, 1603, 1560, 1500, 1445, 1384, 1358, 1306, 1257, 1169, 1087, 1021, 938, 867, 829; ^1H NMR (300 MHz, DMSO-d_6): 8.07 (d, J=8.8 Hz, 2H, Ar–*H*), 7.44-7.56 (m, 3H, Ar–*H*), 7.32–7.35 (m, 2H, Ar–*H*), 7.19 (br s, 2H, NH_2), 6.96 (d, J=8.9 Hz, 2H, Ar–*H*), 6.46 (s, 1H, C*H*), 3.79 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, DMSO-d_6): 169.0 (*C*]O), 158.9 (4-OCH_3–C_6H_4), 157.5 (*C*–NH_2), 138.2 (*C*]CH), 132.4 (C_6H_5), 131.8 (4-OCH_3–C_6H_4), 129.5 (C_6H_5), 128.49 (4-OCH_3–C_6H_4), 128.46 (C_6H_5), 127.7 (C_6H_5), 113.9 (4-OCH_3–C_6H_4), 113.3 (C]*C*H), 55.2 (OCH_3); HRMS (MALDI-TOF/TOF) m/z: $[M+H]^+$ calcd for $C_{17}H_{16}N_3O_2$ 294.1237; Found 294.1227.

*4.5.7. (Z)-4-[(4-Methoxyphenyl)methylidene]-2-amino-1-(4-fluorophenyl)-1H-imidazol-5(4H)-one (**22**).* Isolated as brown crystals (12 mg, 17%). mp 199–200 °C; FTIR (KBr) cm^{-1}: 3394, 1735, 1671, 1628, 1599, 1566, 1507, 1443, 1383, 1370, 1251, 1237, 1169, 1023, 934, 885, 838, 818; ^1H NMR (600 MHz, DMSO-d_6): 8.06 (d, J=8.9 Hz, 2H, Ar–*H*), 7.34–7.41 (m, 4H, Ar–*H*), 7.19 (br s, 2H, NH_2), 6.95 (d, J=8.9 Hz, 2H, Ar–*H*), 6.46 (s, 1H, C*H*), 3.79 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, DMSO-d_6): 169.0 (*C*]O), 161.8 (d, J=245.6 Hz, 4-F–C_6H_4), 158.9 (4-OCH_3–C_6H_4), 157.5 (*C*–NH_2), 138.1 (*C*]CH), 131.8 (4-OCH_3–C_6H_4), 130.2 (d, J=9.1 Hz, 4-F–C_6H_4), 128.7 (d, J=2.9 Hz, 4-F–C_6H_4), 128.4 (4-OCH_3–C_6H_4), 116.3 (d, J=22.8 Hz, 4-F–C_6H_4), 113.8 (4-OCH_3–C_6H_4), 113.3 (C]*C*H), 55.1 (OCH_3); HRMS (MALDI-TOF/TOF) m/z: $[M+H]^+$ calcd for $C_{17}H_{15}FN_3O_2$ 312.1143; Found 312.1146.

*4.5.8. (Z)-4-[(4-Methoxyphenyl)methylidene]-2-amino-1-(4-methoxyphenyl)-1H-imidazol-5(4H)-one (**23**).* Isolated as brown crystals (7 mg, 8%). mp 193–194 °C; FTIR (KBr) cm^{-1}: 3421, 3339, 2919, 1712, 1664, 1621, 1598, 1559, 1507, 1458, 1383, 1366, 1314, 1248, 1171, 1019, 927, 876, 846, 830, 810; ^1H NMR (600 MHz, DMSO-d_6): 8.06 (d, J=8.9 Hz, 2H, Ar–*H*), 7.24 (d, J=8.8 Hz, 2H, Ar–*H*), 7.10 (br s, 2H, NH_2), 7.06 (d, J=8.9 Hz, 2H, Ar–*H*), 6.95 (d, J=8.9 Hz, 2H, Ar–*H*), 6.43 (s, 1H, C*H*), 3.81 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3); ^{13}C NMR (150 MHz, DMSO-d_6): 169.2 (*C*]O), 159.2 (4-OCH_3–C_6H_4), 158.8 (4-OCH_3–C_6H_4), 158.0 (*C*–NH_2), 138.3 (*C*]CH), 131.7 (4-OCH_3–C_6H_4), 129.2 (4-OCH_3–C_6H_4), 128.5 (4-OCH_3–C_6H_4), 124.9 (4-OCH_3–C_6H_4), 114.7 (4-OCH_3–C_6H_4), 113.9 (4-OCH_3–C_6H_4), 113.0 (C]*C*H), 55.4 (OCH_3), 55.1 (OCH_3); HRMS (MALDI-TOF/TOF) m/z: $[M+H]^+$ Anal. Calcd for $C_{18}H_{18}N_3O_3$ 324.1343; Found 324.1340.

*4.5.9. (Z)-4-[(4-Methoxyphenyl)methylidene]-2-amino-1-(4-methylphenyl)-1H-imidazol-5(4H)-one (**24**).* Isolated as brown crystals (13 mg, 15%). mp 210–211 °C; FTIR (KBr) cm^{-1}: 3408, 3339, 2917, 1702, 1664, 1618, 1598, 1560, 1508, 1458, 1370, 1311, 1248, 1169, 1115, 1011, 932, 881, 845, 812; ^1H NMR (600 MHz, DMSO-d_6): 8.06 (d, J=8.8 Hz, 2H, Ar–*H*), 7.33 (d, J=8.1 Hz, 2H, Ar–*H*), 7.20 (d, J=8.2 Hz, 2H, Ar–*H*), 7.12 (br s, 2H, NH_2), 6.95 (d, J=8.9 Hz, 2H, Ar–*H*), 6.44 (s, 1H, C*H*), 3.78 (s, 3H, OCH_3), 2.37 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): 169.0 (*C*]O), 158.9 (4-OCH_3–C_6H_4), 157.7 (*C*–NH_2), 138.3 (*C*]CH), 138.0 (4-CH_3–C_6H_4), 131.7 (4-OCH_3–C_6H_4), 130.0 (4-CH_3–C_6H_4), 129.8 (4-CH_3–C_6H_4), 128.5 (4-OCH_3–C_6H_4), 127.5 (4-CH_3–C_6H_4), 113.8 (4-OCH_3–C_6H_4), 113.1 (C]*C*H), 55.1 (OCH_3), 20.7 (CH_3); HRMS (MALDI-TOF/TOF) m/z: $[M+H]^+$ Anal. Calcd for $C_{18}H_{18}N_3O_2$ 308.1393; Found 308.1383.

*4.5.10. (Z)-4-[(4-Methoxyphenyl)methylidene]-2-amino-1-cyclohexyl-1H-imidazol-5(4H)-one (**25**).* Isolated as brown crystals (23 mg, 26%). mp 176–177 °C; FTIR (KBr) cm^{-1}: 3385, 2918, 2850, 1699, 1666, 1600, 1557, 1506, 1447, 1368, 1281, 1246, 1159, 1082, 1062, 1037, 974, 864, 847, 814; ^1H NMR (600 MHz, DMSO-d_6): 7.98 (d, J=8.8 Hz, 2H, Ar–*H*), 7.35 (br s, 2H, NH_2), 6.91 (d, J=8.9 Hz, 2H, Ar–*H*), 6.27 (s, 1H, C*H*), 3.77–3.82 (m, 1H, cyclohexyl), 3.77 (s, 3H, OCH_3), 2.09–2.15 (m, 2H, cyclohexyl), 1.77–1.79 (m, 2H, cyclohexyl), 1.60–1.62 (m, 3H, cyclohexyl), 1.14–1.32 (m, 3H, cyclohexyl); ^{13}C NMR (150 MHz, DMSO-d_6): 169.8 (*C*]O), 158.62 (4-OCH_3–C_6H_4), 158.56 (*C*–NH_2), 138.6 (*C*]CH), 131.5 (4-OCH_3–C_6H_4), 128.6 (4-

OCH_3–C_6H_4), 113.7 (4-OCH_3–C_6H_4), 111.8 (C]*C*H), 55.1 (OCH_3), 51.6 (cyclohexyl), 29.0 (cyclohexyl), 25.4 (cyclohexyl), 24.5 (cyclohexyl); HRMS (MALDI-TOF/TOF) *m*/*z*: $[M+H]^+$ calcd for $C_{17}H_{22}N_3O_2$ 300.1707; Found 300.1701.

4.6. Synthesis of (*E*)-*N*-(4-((*R*)-4-((*R*)-(4-fluorophenylamino) (4-methoxyphenyl)methyl)-5-oxo-1-(4-cyanophenyl)-1,3-dihydroimidazol-2-ylidene)benzamide (26)

Intermediate **26** was synthesized directly from thiourea **8** (196 mg, 0.70 mmol) and β-lactam (3*R*,4*R*)-**1a** (200 mg, 0.70 mmol) with addition of $HgCl_2$ (189 mg, 0.70 mmol) and Et_3N (194 μL, 1.40 mmol) according to the general procedure for the synthesis of guanidines. The crude product was purified by recrystallization from ethyl acetate and diethyl ether. Intermediate **26** (101 mg, 27%) was obtained as brown crystals. The mother liquor was further subjected to silica gel column chromatography (dichloromethane/hexane/ethyl acetate 3:3:1) to obtain benzoylaminoimidazole **27** as yellow to green powder (128 mg, 44%). mp 171–172 °C; FTIR (KBr) cm^{-1}: 3384, 3277, 1737, 1636, 1587, 1508, 1438, 1338, 1201, 1167, 1023, 828; ^1H NMR (600 MHz, DMSO-d_6): 10.41 (s, 1H, N*H*, imidazolone), 8.00 (d, *J*=8.6 Hz, 2H, Ar–*H*), 7.86–7.87 (m, 2H, Ar–*H*), 7.50–7.52 (m, 1H, Ar–*H*), 7.39 (t, *J*=7.8 Hz, 2H, Ar–*H*), 7.22–7.25 (m, 4H, Ar–*H*), 6.95 (d, *J*=7.7 Hz, 1H, N*H*, side chain), 6.89 (t, *J*=8.9 Hz, 2H, Ar–*H*), 6.82 (d, *J*=8.8 Hz, 2H, Ar–*H*), 6.57–6.59 (m, 2H, Ar–*H*), 5.01 (d, *J*=2.0 Hz, 1H, C*H*, imidazolone), 4.96 (d, *J*=4.3 Hz, 1H, C*H*, side chain), 3.65 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, DMSO-d_6): 176.1 (N-(*C*]O)C_6H_5), 170.4 (*C*]O, imidazolone), 158.9 (4-OCH_3–C_6H_4), 158.7 (*C*]N), 154.6 (d, *J*=232.1 Hz, 4-F–C_6H_4), 143.5 (4-F–C_6H_4), 136.4 (C_6H_5), 135.7 (4-CN–C_6H_4), 132.9 (C_6H_5), 132.2 (C_6H_5), 128.9 (C_6H_5), 128.9 (4-CN–C_6H_4), 128.3 (4-CN–C_6H_4), 128.2 (4-OCH_3–C_6H_4), 128.1 (4-OCH_3–C_6H_4), 118.3 (4-CN–C_6H_4), 115.4 (d, *J*=22.1 Hz, 4-F–C_6H_4), 113.9 (d, *J*=7.3 Hz, 4-F–C_6H_4), 113.5 (4-OCH_3–C_6H_4), 111.0 (*C*≡N), 63.2 (*C*H–CH, imidazolone), 58.1 (CH–*C*H, side chain), 55.0 (OCH_3); HRMS (MALDI-TOF/TOF) *m*/*z*: [M–e^-] Anal. Calcd for $C_{31}H_{24}FN_5O_3$ 533.1858; Found 533.1865.

4.7. Synthesis of 27 and 28

Benzoylaminoimidazolone **27** and 2-aminoimidazolone **28** were synthesized according to the general procedure for the preparation of benzoylaminoimidazolones and 2-aminoimidazolones from intermediate **26**.

*4.7.1. (E)-N-((Z)-4-((4-Methoxyphenyl)methylidene)-5-oxo-1-(4-cyanophenyl)-1,3-dihydroimidazol-2-ylidene)benzamide (**27**).* Isolated as yellow to green powder (95 mg, 85%). mp 248–249 °C; FTIR (KBr) cm^{-1}: 3459, 2231, 1746, 1634, 1588, 1514, 1443, 1395, 1333, 1268, 1243, 1174, 1076, 1014, 826; ^1H NMR (600 MHz, $CDCl_3$): 12.02 (br s, 1H, N*H*), 8.12–8.13 (m, 2H, Ar–*H*), 7.86 (d, *J*=8.7 Hz, 2H, Ar–H), 7.82 (d, *J*=8.7 Hz, 2H, Ar–H), 7.52–7.54 (m, 3H, Ar–*H*), 7.42 (t, *J*=7.7 Hz, 2H, Ar–*H*), 7.07 (d, *J*=8.7 Hz, 2H, Ar–*H*), 7.00 (s, 1H, C*H*), 3.90 (s, 3H, OCH_3); ^{13}C NMR (150 MHz, $CDCl_3$): 179.4 (N-(*C*]O)C_6H_5), 162.0 (*C*]O, imidazolone), 161.5 (4-OCH_3–C_6H_4), 155.7 (*C*]N), 136.4 (C_6H_5), 135.8 (4-CN–C_6H_4), 133.1 (C_6H_5), 132.9 (4-CN–C_6H_4), 131.5 (C_6H_5), 129.9 (C_6H_5), 128.5 (4-OCH_3–C_6H_4), 127.7 (4-CN–C_6H_4), 125.2 (*C*]CH), 122.5 (4-OCH_3–C_6H_4), 118.3 (4-CN–C_6H_4), 117.0 (C]*C*H), 115.5 (4-OCH_3–C_6H_4), 112.2 (*C*≡N), 55.7 (OCH_3); HRMS (MALDI-TOF/TOF) *m*/*z*: $[M+H]^+$ Anal. Calcd for $C_{25}H_{19}N_4O_3$ 423.1451; Found 423.1435.

*4.7.2. (Z)-4-[(4-Methoxyphenyl)methylidene]-2-amino-1-(4-cyanophenyl)-1H-imidazol-5(4H)-one (**28**).* Isolated as brown crystals (1 mg, ~1%). mp 160–162 °C; FTIR (KBr) cm^{-1}: 3403, 2918, 2852, 2231, 1718, 1675, 1627, 1600, 1508, 1450, 1368, 1251, 1165, 1031, 821; ^1H NMR (600 MHz, DMSO-d_6): 8.07 (d, *J*=8.7 Hz, 2H, Ar–*H*); 8.00 (d, *J*=8.4 Hz, 2H, Ar–*H*), 7.58 (d, *J*=8.5 Hz, 2H, Ar–*H*), 7.28 (br s, 2H, NH_2), 6.96 (d, *J*=8.7 Hz, 2H, Ar–*H*), 6.50 (s, 1H, C*H*), 3.79 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, DMSO-d_6): 168.4 (*C*]O), 159.0 (4-OCH_3–C_6H_4), 156.6 (*C*–NH_2), 137.7 (*C*]CH), 136.7 (4-CN–C_6H_4), 133.5 (4-CN–C_6H_4), 131.9 (4-OCH_3–C_6H_4), 128.6 (4-CN–C_6H_4), 128.3 (4-OCH_3–C_6H_4), 118.4 (4-CN–C_6H_4), 113.96 (C]*C*H), 113.89 (4-OCH_3–C_6H_4), 110.9 (*C*≡N), 55.2 (OCH_3); HRMS (MALDI-TOF/TOF) *m*/*z*: $[M+H]^+$ Anal. Calcd for $C_{18}H_{15}N_4O_2$ 319.1189; Found 319.1193.

4.8. Synthesis of (*E*)-*N*-((*Z*)-4-((4-methoxyphenyl)methylidene)-5-oxo-1-(4-nitrophenyl)-1,3-dihydroimidazol-2-ylidene)benzamide (30)

Benzoylaminoimidazol **30** was synthesized from thiourea **8** (75 mg, 0.25 mmol) and β-lactam **1a** (72 mg, 0.25 mmol) with addition of $HgCl_2$ (68 mg, 0.25 mmol) and Et_3N (70 μL, 0.50 mmol) according to the procedure for the preparation of guanidines. The reaction mixture was filtered and evaporated to dryness, after which the filtrate was dissolved in methanol (10 mL) and K_2CO_3 (69 mg, 0.50 mmol) added to obtain benzoylaminoimidazolone **30**. The crude product was purified by silica gel chromatography (dichloromethane/hexane/ethyl acetate 3:3:1). Product **30** (68 mg, 62%) was obtained as yellow to green powder. mp 285–286 °C; FTIR (KBr) cm^{-1}: 3463, 1746, 1636, 1600, 1587, 1521, 1347, 1325, 1267, 1242, 1176, 1027, 1010, 854, 828, 817; ^1H NMR (300 MHz, DMSO-d_6): 11.75 (br s, 1H, N*H*), 8.44 (d, *J*=8.4 Hz, 2H, Ar–*H*), 7.90–8.03 (m, 4H, Ar–*H*), 7.70 (br s, 2H, Ar–*H*), 7.55–7.60 (m, 1H, Ar–*H*), 7.46 (t, *J*=7.4 Hz, 2H, Ar–*H*), 7.16 (d, *J*=8.5 Hz, 2H, Ar–*H*), 7.02 (s, 1H, C*H*), 3.86 (s, 3H, OCH_3); Satisfactory ^{13}C NMR could not be obtained due to very low solubility of the compound; Anal. Calcd for $C_{24}H_{18}N_4O_5$: C, 65.15; H, 4.10; N, 12.66. Found: C, 64.94; H, 4.04; N, 12.75.

4.9. Synthesis of (*E*)-*N*-(4-(4-fluorophenylamino) (4-methoxyphenyl)methyl-5-oxo-1-phenyl-1,3-dihydroimidazol-2-ylidene)benzamide (31)

To a solution of guanidine **9a** (30 mg, 0.06 mmol) in CH_3CN was added K_2CO_3 (16 mg, 0.12 mmol). The reaction mixture was stirred overnight at room temperature and the reaction mixture evaporated to dryness. Distilled water was added and the resulting mixture extracted with dichloromethane (3×30 mL). Combined organic layers were dried on Na_2SO_4, filtered and evaporated. The crude product was purified by silica gel column chromatography (dichloromethane/hexane/ethyl acetate 3:3:1) and preparative thin-layer chromatography (hexane/ethyl acetate 2:1). A mixture of isomers of intermediate **31** (7 mg, 23%) was obtained as brown oil. ^1H NMR (300 MHz, $CDCl_3$): 9.75 (br s, 1H, N*H*, imidazolone), 9.68 (br s, 1H, N*H*, imidazolone), 8.01–8.05 (m, 4H, Ar–*H*), 7.38–7.49 (m, 10H, Ar–*H*), 7.32–7.36 (m, 4H, Ar–*H*), 7.28 (d, *J*=8.7 Hz, 2H, Ar–*H*), 7.20–7.21 (m, 2H, Ar–*H*), 7.04–7.06 (m, 2H, Ar–*H*), 6.87–6.91 (m, 6H, Ar–*H*), 6.83 (t, *J*=8.7 Hz, 2H, Ar–*H*), 6.67–6.69 (m, 2H, Ar–*H*), 6.59–6.61 (m, 2H, Ar–*H*), 5.05 (dd, *J*=10.3 Hz, 3.0 Hz, 1H, C*H*, side chain), 4.88 (d, *J*=4.9 Hz, 1H, C*H*, side chain), 4.79–4.81 (d, *J*=5.0 Hz, 1H, C*H*, imidazolone; 1H, N*H*, side chain), 4.73 (d, *J*=3.3 Hz, 1H, C*H*, imidazolone), 4.51 (br s, 1H, N*H*, side chain), 3.77 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): 179.6 (N-(*C*]O)C_6H_5), 179.5 (N-(*C*]O)C_6H_5), 171.5 (*C*]O, imidazolone), 171.1 (*C*]O, imidazolone), 160.5 (d, *J*=242.5 Hz, 4-F–C_6H_4), 160.3 (4-OCH_3–C_6H_4), 157.9 (*C*]N), 147.1 (C_6H_5), 142.1 (d, *J*=2.1 Hz, 4-F–C_6H_4), 136.7 (C_6H_5), 136.6 (C_6H_5), 132.6 (C_6H_5), 132.5 (C_6H_5), 129.89 (C_6H_5), 129.85 (C_6H_5), 129.04 (C_6H_5), 128,99 (C_6H_5), 128.86 (C_6H_5), 128.81 (4-OCH_3-C_6H_4), 128.80 (C_6H_5), 128.28 (C_6H_5), 128.24 (C_6H_5), 128.1 (4-OCH_3–C_6H_4),

128.0 (4-OCH_3–C_6H_4), 127.32 (C_6H_5), 127.29 (C_6H_5), 116.1 (d, J=22.1 Hz, 4-F–C_6H_4), 116.00 (d, J=22.7 Hz, 4-F–C_6H_4), 115.92 (d, J=6.9 Hz, 4-F–C_6H_4), 115.85 (d, J=7.1 Hz, 4-F–C_6H_4), 114.8 (4-OCH_3–C_6H_4), 114.0 (4-OCH_3–C_6H_4), 62.8 (*C*H–CH, imidazolone), 61.4 (CH–*C*H, imidazolone), 60.5 (CH–*C*H, side chain), 58.6 (CH–*C*H, side chain), 55.5 (OCH_3); HRMS for $C_{30}H_{26}FN_4O_3$ (M_r=508.5429): calcd m/z $[M+H]^+$ 509.1983, found 509.1976.

4.10. Computational details

Due to high conformational flexibility of phenyl groups attached to central moiety, we used simplified model structures where two phenyl groups (not directly participating in the reaction of interest) were cut out from the original structure and replaced with methyl groups. Geometry optimizations with all model structures were performed at B3LYP/6–31+G(d) level of theory. On top of that, single point MP2/6–311++G(d,p)//B3LYP/6–31+G(d) energy calculations were executed.[39–41] Solvation effects during geometry optimization and single point calculations were taken into account by SMD solvation model[42] with methanol as solvent. Vibrational analysis confirmed the stationary nature of minima at the potential energy surface. Transition states were optimized by using QST3 method[43] and their existence was verified by vibrational analysis and identified by presence of single imaginary frequency corresponding to bond breaking/forming process. Gibbs free energy is calculated as a sum of single point electronic energy, thermal correction to Gibbs free energy and energy of solvation at 298 K. All calculations were performed with Gaussian09 suite of codes.[44]

4.11. Antiproliferative evaluation assay

HCT116 and H460 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) (Sigma, USA) containing 100 units/mL penicillin and 100 μg/mL streptomycin supplemented with 10% FBS (Sigma, USA). and 2 mM L-glutamine and were grown in a humidified 5% CO_2 incubator at 37 °C. For the experiment, cells were seeded in 96-well microtiter plates at 1.5×10^4 cells/mL (150 μL/well). After 24 h tested compounds (20 mM Stock in DMSO) were added to obtain consecutive 10-fold dilutions (10^{-8} to 10^{-4} mol/L) and further incubated for 72 h. Antiproliferative activity was assessed using MTT cell proliferation assay (Promega, USA) following a standard protocol.[45,46] The absorbance (A) was measured on a microplate reader at 570 nm. The results are expressed as IC_{50}, the concentration causing 50% inhibition of growth. The IC_{50} values for each compound were calculated from dose-response curves using linear regression analysis. The experiments were performed in quadruplicates and the results represent a mean of three independent experiments.

Acknowledgements

This research was funded by the Croatian Ministry of Science, Education and Sports (Programs 098-0982915-2948 and 098-0982522-2525). M. V. acknowledges support from the Croatian Academy of Science and Arts, M. D. from the Croatian Ministry of Science, Education and Sports (Program 119-1193079-1332), M. K. from the EU 7th Framework Program for Research and Technological Development (316289-InnoMol, FP7-REGPOT-2012-2013-1) and S. H. from Swiss NSF-SCOPES project.

Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2015.10.048.

References and notes

1. Alcaide, B.; Almendros, P.; Aragoncillo, C. *Chem. Rev.* **2007**, *107*, 4437–4492.
2. Palomo, C.; Oiarbide, M. In *Topics in Heterocyclic Chemistry (Heterocyclic Scaffolds I; β-Lactams)*; Maes, B. U. W., Banik, B. K., Eds.; Springer: Berlin Heidelberg, 2010; 22, pp 211–259.
3. Alcaide, B.; Aly, M. F.; Rodríguez, C.; Rodríguez-Vicente, A. *J. Org. Chem.* **2000**, *65*, 3453–3459.
4. Alcaide, B.; Almendros, P.; Redondo, M. C. *Chem. Comm.* **2006**, 2616–2618.
5. Singh, P.; Mehra, V.; Anand, A.; Kumar, V.; Mahajan, M. P. *Tetrahedron Lett.* **2011**, *52*, 5060–5063.
6. Mehra, V.; Singh, P.; Kumar, V. *Tetrahedron* **2012**, *68*, 8395–8402.
7. Fu, X.; Tan, C.-H. *Chem. Commun.* **2011**, 8210–8222.
8. Taylor, J. E.; Bull, S. D.; Williams, J. M. *J. Chem. Soc. Rev.* **2012**, *41*, 2109–2121.
9. Maji, B.; Stephenson, D. S.; Mayr, H. *ChemCatChem* **2012**, *4*, 993–999.
10. Carmely, S.; Ilan, M.; Kashman, Y. *Tetrahedron* **1989**, *45*, 2193–2200.
11. Chan, G. W.; Mong, S.; Hemling, M. E.; Freyer, A. J.; Offen, P. H.; DeBrosse, C. W.; Sarau, H. M.; Westley, J. W. *J. Nat. Prod.* **1993**, *56*, 116–121.
12. Crews, P.; Clark, D. P.; Tenney, K. *J. Nat. Prod.* **2003**, *66*, 177–182.
13. Urban, S.; de Almeida Leone, P.; Carroll, A. R.; Fechner, G. A.; Smith, J.; Hooper, J. N. A.; Quinn, R. J. *J. Org. Chem.* **1999**, *64*, 731–735.
14. Sullivan, J. D.; Giles, R. L.; Looper, R. E. *Curr. Bioact. Compd.* **2009**, *5*, 39–78.
15. Stachel, S. J.; Coburn, C. A.; Rush, D.; Jones, K. L. G.; Zhu, H.; Rajapakse, H.; Graham, S. L.; Simon, A.; Holloway, M. K.; Allison, T. J.; Munshi, S. K.; Espeseth, A. S.; Zuck, P.; Colussi, D.; Wolfe, A.; Pietrak, B. L.; Lai, M.-T.; Vacca, J. P. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2977–2980.
16. Hills, I. D.; Holloway, M. K.; de León, P.; Nomland, A.; Zhu, H.; Rajapakse, H.; Allison, T. J.; Munshi, S. K.; Colussi, D.; Pietrak, B. L.; Toolan, D.; Haugabook, S. J.; Graham, S. L.; Stachel, S. J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4993–4995.
17. Chiriano, G.; De Simone, A.; Mancini, F.; Perez, D. I.; Cavalli, A.; Bolognesi, M. L.; Legname, G.; Martinez, A.; Andrisano, V.; Carloni, P.; Roberti, M. *Eur. J. Med. Chem.* **2012**, *48*, 206–213.
18. Swahn, B.-M.; Holenz, J.; Kihlström, J.; Kolmodin, K.; Lindström, J.; Plobeck, N.; Rotticci, D.; Sehgelmeble, F.; Sundström, M.; von Berg, S.; Fälting, J.; Georgievska, B.; Gustavsson, S.; Neelissen, J.; Ek, M.; Olsson, L.-L.; Berg, S. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1854–1859.
19. Ilies, M.; Di Costanzo, L.; North, M. L.; Scott, J. A.; Christianson, D. W. *J. Med. Chem.* **2010**, *53*, 4266–4276.
20. Subtel'na, I.; Atamanyuk, D.; Szymańska, E.; Kieć-Kononowicz, K.; Zimenkovsky, B.; Vasylenko, O.; Gzella, A.; Lesyk, R. *Bioorg. Med. Chem.* **2010**, *18*, 5090–5102.
21. Lawson, A. *J. Chem. Soc.* **1956**, 307–310.
22. Lancini, G. C.; Lazzari, E. *J. Heterocycl. Chem.* **1966**, *3*, 152–154.
23. Little, T. L.; Webber, S. E. *J. Org. Chem.* **1994**, *59*, 7299–7305.
24. Patel, J.; Pelloux-Léon, N.; Minassian, F.; Valée, Y. *Tetrahedron Lett.* **2006**, *47*, 5561–5563.
25. Yamaguchi, J.; Seiple, I. B.; Young, I. S.; O'Malley, D. P.; Maue, M.; Baran, P. S. *Angew. Chem., Int. Ed.* **2008**, *47*, 3578–3580.
26. Baran, P. S.; Zografos, A. L.; O'Malley, D. P. *J. Am. Chem. Soc.* **2004**, *126*, 3726–3727.
27. Claramunt, R. M.; Granados, R.; Repolles, M. C. *Anal. Quim.* **1975**, *71*, 206–207.
28. Molina, P.; López-Leonardo, C.; Llamas-Botía, J.; Foces-Foces, C.; Llamas-Saiz, A. L. *Synthesis* **1995**, *4*, 449–452.
29. Roué, N.; Bergman, J. *Tetrahedron* **1999**, *55*, 14729–14738.
30. Thangavel, N.; Murgesh, K. *Asian J. Chem.* **2005**, *17*, 2769–2772.
31. Ermolat'ev, D. S.; Babaev, E. V.; Van der Eycken, E. V. *Org. Lett.* **2006**, *8*, 5781–5784.
32. Debdab, M.; Renault, S.; Lozach, O.; Meijer, L.; Paquin, L.; Carreaux, F.; Bazureau, J.-P. *Eur. J. Med. Chem.* **2010**, *45*, 805–810.
33. Chérouvrier, J.-R.; Carreaux, F.; Bazureau, J. P. *Tetrahedron Lett.* **2002**, *43*, 3581–3584.
34. Ojima, I.; Habuš, I. *Tetrahedron Lett.* **1990**, *31*, 4289–4292.
35. Poljak, T.; Molčanov, K.; Višnjevac, A.; Habuš, I.; Kovač, V.; Rapić, V. *J. Mol. Struct.* **2005**, *751*, 60–64.
36. Cunha, S.; Costa, M. B.; Napolitano, H. B.; Lariucci, C.; Vencato, I. *Tetrahedron* **2001**, *57*, 1671–1675.
37. Crowell, T. I.; Kemp, R. T.; Lutz, R. E.; Wall, A. A. *J. Am. Chem. Soc.* **1968**, *90*, 4638–4643.
38. Spillane, W. J.; McGrath, P.; Brack, C.; O'Byrne, A. B. *J. Org. Chem.* **2001**, *66*, 6313–6316.
39. Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B Condens. Matter Mater. Phys.* **1988**, *37*, 785–789.
40. Becke, A. D. *Phys. Rev. A* **1988**, *38*, 3098–3100.
41. Pople, J. A.; Krishnan, R.; Schlegel, H. B.; Binkley, J. S. *Int. J. Quantum Chem.* **1979**, *16*, 225–241.
42. Marenich, A. V.; Cramer, C. J.; Truhlar, D. G. *J. Phys. Chem. B* **2009**, *113*, 6378–6396.
43. Peng, C.; Bernhard Schlegel, H. *Isr. J. Chem.* **1993**, *33*, 449–454.
44. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery Jr, J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J.; Brothers, E. N.; Kudin,

K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, N. J.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian, Inc.: Wallingford, CT, USA, **2009**.

45. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55–63.
46. Jarak, I.; Kralj, M.; Piantanida, I.; Šuman, L.; Žinić, M.; Pavelić, K.; Karminski-Zamola, G. *Bioorg. Med. Chem.* **2006**, *14*, 2859–3286.