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# Synthesis, docking and evaluation of *in vitro* anti-inflammatory activity of novel morpholine capped $\beta$ -lactam derivatives

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## Abstract:

This study reports the synthesis and biological investigation of three series of novel  $\beta$ -lactams bearing a morpholine substituent of the nitrogen center. These products were synthesized via Staudinger's [2+2]-ketene-imine cycloaddition reaction. The cycloadducts were fully characterized by spectral data, including <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, IR, mass spectroscopy and elemental analyses, and then evaluated for anti-inflammatory activity. A number of the derivatives demonstrated higher therapeutic ratios than dexamethasone and corticosteroid medication. *In silico* molecular docking experiments showed a good correlation between the experimental activity and the calculated binding affinity to human inducible nitric oxide synthase, the enzymatic target for the anti-inflammatory response.

**Keywords:** 2-azetidinone, Staudinger reactions, morpholine, anti-inflammatory, NOS, *In silico*

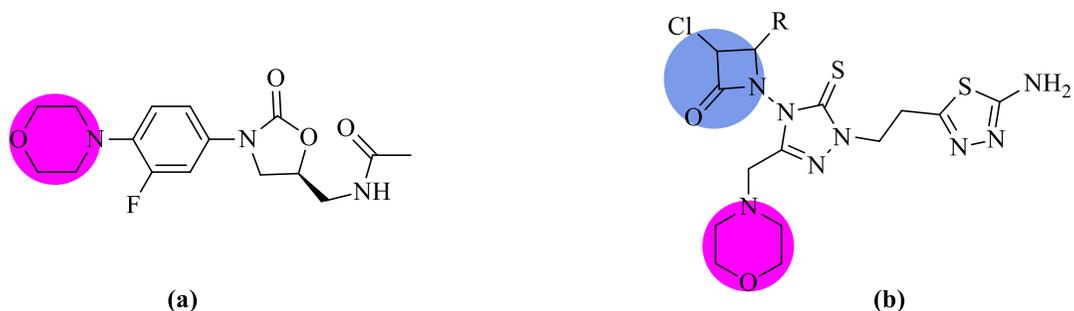
## Introduction:

Inflammation is one of the most important defenses of the body against tissue damage or infection. However, chronic inflammation may lead to disease, such as arthritis, atherosclerosis, psoriasis, cancer, infections, asthma, etc. [1,2] Therefore, treatment of inflammation is a high priority topic for researchers over the past decades. [3] Inflammation as a biological signal of the immune system can be caused by different factors such as pathogens, damaged cells and toxic compounds. [4] Abnormal activation of certain enzymes such as inducible nitric oxide synthase (iNOS) [5] is one reason for inflammation-related disease and inhibition of iNOS may exert anti-inflammatory effects. [6] Therefore, development of anti-inflammatory chemicals is still of great importance.

$\beta$ -Lactams are among the most important classes of organic compounds in the last century due to their diverse biological activities, [7,8] including their well-known antibacterial properties, as well as cholesterol absorption inhibition, [9,10] cysteine protease inhibition, [11] tryptase and chymase inhibition, [12] and anticancer, [13,14] antifungal, [15,16] antimalarials, [17,18] anti-HIV, [19] antidiabetic, [20] antioxidant, [21] and anti-inflammatory activities. [22,23] Kumar *et.al.* synthesized a series of quinazolinone  $\beta$ -lactam conjugates and investigated their anti-inflammatory activities. [22] Recently, our laboratories have prepared chromeno  $\beta$ -lactam hybrids and have examined their anti-inflammatory properties. [23]

Another class of nitrogen ring compounds are the morpholine derivatives, often used as the building blocks, [24] selective norepinephrine reuptake inhibitors [25] and dopamine D4 receptor ligands. [26] Moreover, they have been shown anti-inflammatory, [27,28] antiparasitic, [29] antimicrobial, [30,31] antitumor, [32] and antioxidant activities. [33,34] Linezolid is an example of a commercially important antibacterial agent possessing a morpholine ring (Scheme 1). [35] Recently, Cebeci *et.al.* have reported  $\beta$ -lactam derivatives containing a morpholine moiety (Scheme 1) that possess antimicrobial, antioxidant, antitubercular, and antiurease activities, as well as acetylcholinesterase inhibitory properties. [36]

Our interest in new structural variants of biologically active  $\beta$ -lactams has led us to study hybridized systems, wherein the  $\beta$ -lactam core is combined with other relevant heterocycles. In this current study, we detail our studies on the synthesis and anti-inflammatory properties of  $\beta$ -lactam-morpholine hybrids.

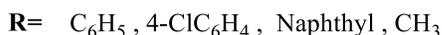
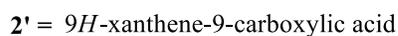
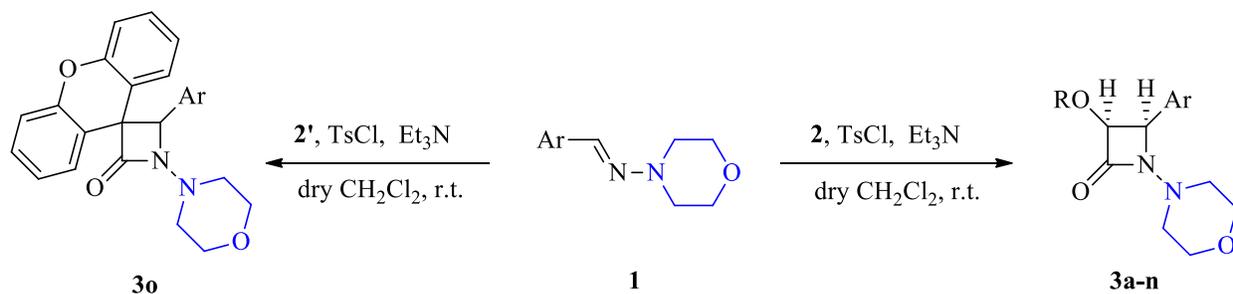


**Scheme 1.** Structures of (a) linezolid and (b)  $\beta$ -lactam derivatives bearing a morpholine ring [36]

## Results and discussion

### Chemistry

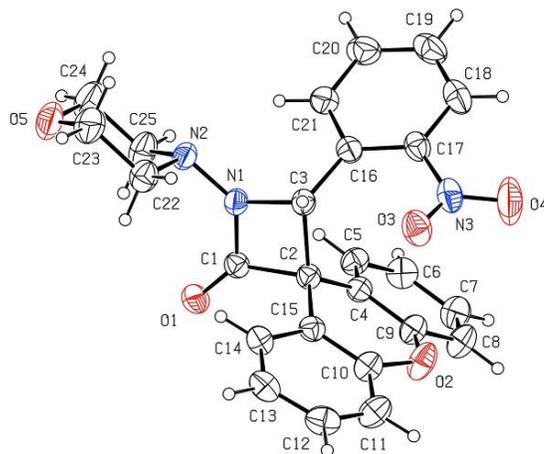
Morpholine-substituted  $\beta$ -lactams **3a–o** were synthesized by the [2+2]-imine-ketene cycloaddition (Staudinger reaction) of N-morpholinohydrazone **1** with ketene precursors **2** and **2'** in the presence of *p*-toluenesulfonyl chloride and triethylamine in anhydrous dichloromethane (Scheme 2; Table 1).



**Scheme 2.** General synthetic pathway for the synthesis of  $\beta$ -lactams **3a–o**

These resulting  $\beta$ -lactam adducts were purified by silica gel chromatography, then structurally characterized by spectral and elemental analyses. It is noteworthy that all the  $\beta$ -lactams **3a–n** possess the *cis* relative configuration as determined from the observed coupling constants of

the H-3 and H-4 protons of the  $\beta$ -lactam ring ( $J = 4.6\text{--}5.1$  Hz) in the  $^1\text{H-NMR}$  spectra. Spiro- $\beta$ -lactam **3o** showed a single peak at 5.38 Hz for the sole  $\beta$ -lactam ring hydrogen. X-ray crystallographic analysis of **3o** [37] revealed that the  $\beta$ -lactam ring is nearly planar and the morpholine ring adopts a chair conformation (Fig. 1).



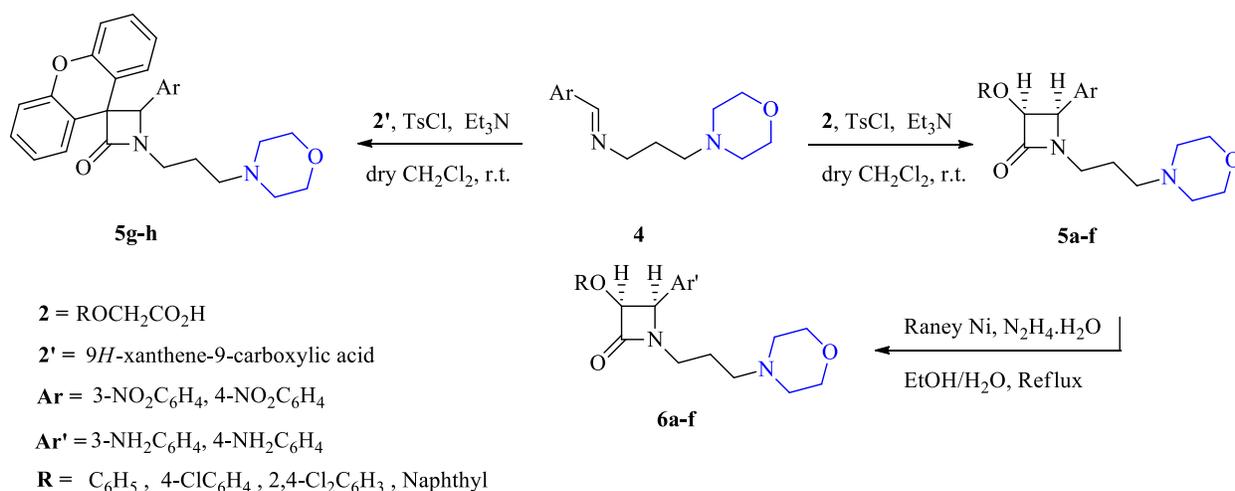
**Fig. 1.** X-Ray structure analysis of compound **3o**.

**Table 1.** Isolated yield of monocyclic  $\beta$ -lactams **3a-o**.

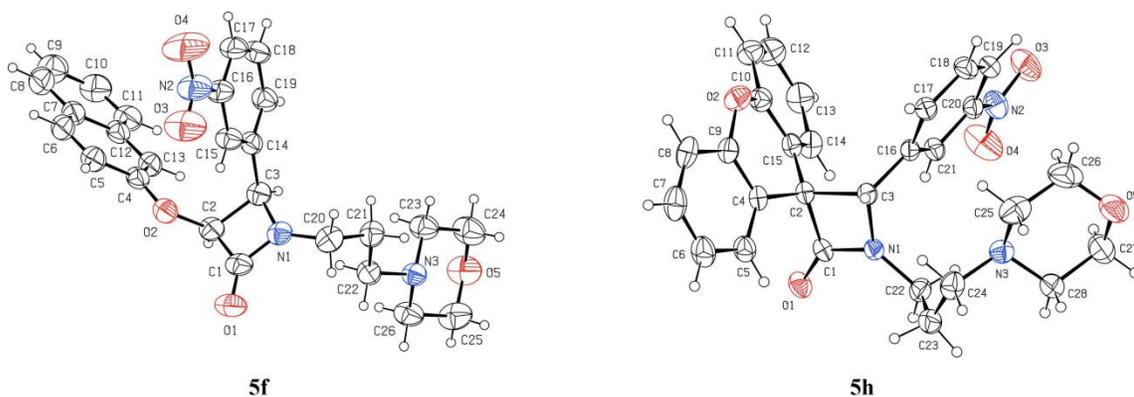
Entry	Ar	R	yield (%)	Entry	Ar	R	yield (%)
<b>3a</b>	4-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	C <sub>6</sub> H <sub>5</sub>	32	<b>3i</b>	2-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	Naphthyl	38
<b>3b</b>	4-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	4-ClC <sub>6</sub> H <sub>4</sub>	35	<b>3j</b>	2-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	CH <sub>3</sub>	64
<b>3c</b>	4-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	Naphthyl	38	<b>3k</b>	4-CNC <sub>6</sub> H <sub>4</sub>	C <sub>6</sub> H <sub>5</sub>	35
<b>3d</b>	4-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	CH <sub>3</sub>	45	<b>3l</b>	4-CNC <sub>6</sub> H <sub>4</sub>	4-ClC <sub>6</sub> H <sub>4</sub>	29
<b>3e</b>	3-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	C <sub>6</sub> H <sub>5</sub>	25	<b>3m</b>	4-CNC <sub>6</sub> H <sub>4</sub>	Naphthyl	49
<b>3f</b>	3-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	CH <sub>3</sub>	43	<b>3n</b>	4-CNC <sub>6</sub> H <sub>4</sub>	CH <sub>3</sub>	36
<b>3g</b>	2-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	C <sub>6</sub> H <sub>5</sub>	36	<b>3o</b>	2-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	-	41
<b>3h</b>	2-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	4-ClC <sub>6</sub> H <sub>4</sub>	23				

In an earlier paper of our research group morpholine  $\beta$ -lactams conjugates linked via propyl reported as antifungal agents. [38] Therefore, to investigate the influence of propyl linkage between  $\beta$ -lactam and morpholine rings,  $\beta$ -lactams **5a-h** were synthesized via Staudinger reactions of the corresponding imines **4** with different substituted acetic acids **2** or **2'** in the presence of *p*-toluenesulfonyl chloride and trimethylamine. Isolated product yields were moderate to good (Scheme 3; Table 2). All the products were characterized by spectral and elemental analyses. The

*cis* stereochemistry of monocyclic  $\beta$ -lactams **5a–f** was deduced from the analysis of their  $^1\text{H-NMR}$  spectra. The spiro- $\beta$ -lactams **5g** and **5h** showed a single peak for H-4 at 4.73 and 4.72 Hz, respectively. The X-ray crystallographic structures of **5f** [39] and **5h** [40] revealed a chair conformation for the morpholine ring and a nearly planar  $\beta$ -lactam ring in **5h** (Fig. 2). The aryl nitro substituent in compounds **5a–f** could be selectively reduced with Raney nickel and aqueous hydrazine to give aminoaryl  $\beta$ -lactams **6a–f** (Table 2). The *cis* ring stereochemistry of **6a–f** was confirmed by  $^1\text{H-NMR}$  spectroscopy.



**Scheme 3.** General synthetic pathway for the synthesis of  $\beta$ -lactams **5a–h** and **6a–f**.



**Fig. 2.** X-ray crystal structures of compounds **5f** and **5h**.

**Table 2.** Isolated yield of monocyclic  $\beta$ -lactams **5a–h** and **6a–f**.

Entry	Ar	R	yield (%)	Entry	Ar	R	yield (%)
<b>5a</b> <sup>a</sup>	4-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	C <sub>6</sub> H <sub>5</sub>	76	<b>5h</b>	3-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	-	59
<b>5b</b>	4-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	4-ClC <sub>6</sub> H <sub>4</sub>	74	<b>6a</b>	4-NH <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	C <sub>6</sub> H <sub>5</sub>	96
<b>5c</b>	4-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	2,4-Cl <sub>2</sub> C <sub>6</sub> H <sub>3</sub>	63	<b>6b</b>	4-NH <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	4-ClC <sub>6</sub> H <sub>4</sub>	92
<b>5d</b>	4-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	Naphthyl	79	<b>6c</b>	4-NH <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	2,4-Cl <sub>2</sub> C <sub>6</sub> H <sub>3</sub>	67
<b>5e</b> <sup>a</sup>	3-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	C <sub>6</sub> H <sub>5</sub>	63	<b>6d</b>	4-NH <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	Naphthyl	79
<b>5f</b>	3-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	Naphthyl	63	<b>6e</b>	3-NH <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	C <sub>6</sub> H <sub>5</sub>	68
<b>5g</b>	4-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	-	71	<b>6f</b>	3-NH <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	Naphthyl	59

<sup>a</sup> previously reported synthesis [38]

### Anti-Inflammatory Assay

Our interest in these morpholine- $\beta$ -lactam hybrids was with respect to possible anti-inflammatory activity, a program our laboratories initiated recently. Anti-inflammatory activity was evaluated through the inhibition of nitric oxide production by LPS-stimulated mouse macrophages. Nitric oxide (NO) is a short-lived molecule, which is released from a variety of cells in response to different pathologic stimuli. NO exerts modulatory activity under a variety of inflammatory conditions, and may act as a vasodilator and a platelet inhibitor, and prevent neutrophil adhesion. As such, NO plays a protective role during infections and other stresses to the immune system.

In the anti-inflammatory assay, inflammation reactions are induced by a simulated microbial infection in macrophage cultures. In response to the microbial infection, a strong production of NO is achieved by macrophages in the culture medium. The anti-inflammatory activity is based on the capacity of compounds to inhibit inflammation reactions, and then NO release in the culture medium. The anti-inflammatory potential is evaluated by calculating, for each compound, the ratio between the anti-inflammatory activity and the cytotoxicity.

Results of the anti-inflammatory assays are reported in Table 3. Nine compounds, namely **3c**, **3h**, **3k**, **3o**, **5c**, **5d**, **5f**, **6d**, and **6f**, presented excellent IC<sub>50</sub> values for NO release, similar or better than the dexamethasone corticosteroid medication. The IC<sub>50</sub> values for cell viability were also measured, in order to determine anti-inflammatory ratios. From this, we observed that morpholine- $\beta$ -lactams **3e**, **3h**, **3k**, **5c**, **5f**, **6c**, **6d**, and **6f** afford improved anti-inflammatory ratios to that of dexamethasone. Inspection of compounds **5c**, **5d**, **5f**, **6c**, **6d**, and **6f** indicates that the phenyl ring substituent (NO<sub>2</sub> versus NH<sub>2</sub>) plays an important role on anti-inflammatory activities.

For instance, **6f** bearing an NH<sub>2</sub> at the meta position provides a better anti-inflammatory ratio (99%) compared to its para isomer **6d** (55%).

**Table 3.** Anti-inflammatory activity of derivatives **3a-o**, **5a-h**, and **6a-f**.

Entry	IC <sub>50</sub> -NO release (μM)	IC <sub>50</sub> -cell viability	anti-inflammatory ratio
		(μM)	
<b>3a</b>	35.41 ± 2.04	256.83 ± 12.14	7
<b>3b</b>	10.67 ± 1.22	267.48 ± 11.65	26
<b>3c</b>	9.90 ± 2.18	147.25 ± 1.65	14
<b>3d</b>	45.51 ± 3.13	275.28 ± 13.24	6
<b>3e</b>	13.04 ± 2.47	>500	<b>&gt;38</b>
<b>3f</b>	30.87 ± 1.99	193.17 ± 9.41	6
<b>3g</b>	14.76 ± 1.52	293.84 ± 8.47	21
<b>3h</b>	7.92 ± 1.07	>500	<b>&gt;62</b>
<b>3i</b>	21.07 ± 1.95	263.34 ± 10.59	12
<b>3j</b>	57.45 ± 2.34	114.15 ± 1.66	2
<b>3k</b>	4.57 ± 0.81	204.11 ± 12.65	<b>51</b>
<b>3l</b>	ND	ND	ND
<b>3m</b>	20.88 ± 3.21	>500	>25
<b>3n</b>	91.88 ± 2.64	264.17 ± 8.74	3
<b>3o</b>	2.93 ± 0.66	14.19 ± 0.88	5
<b>5a</b>	27.63 ± 2.37	>500	>18
<b>5b</b>	17.18 ± 1.56	20.41 ± 1.56	1
<b>5c</b>	4.14 ± 0.63	290.16 ± 12.47	<b>72</b>
<b>5d</b>	5.06 ± 0.94	69.88 ± 4.68	14
<b>5e</b>	28.73 ± 1.89	157.08 ± 5.64	5
<b>5f</b>	5.13 ± 0.95	257.21 ± 9.51	<b>51</b>
<b>5g</b>	60.41 ± 8.34	323.39 ± 12.14	5
<b>5h</b>	12.37 ± 2.08	148.24 ± 1.11	12
<b>6a</b>	70.44 ± 5.49	129.90 ± 10.23	2
<b>6b</b>	17.33 ± 2.91	33.68 ± 1.26	2
<b>6c</b>	14.46 ± 1.34	>500	<b>&gt;35</b>
<b>6d</b>	6.53 ± 1.24	328.61 ± 15.63	<b>55</b>
<b>6e</b>	24.73 ± 1.26	272.92 ± 8.29	11
<b>6f</b>	5.73 ± 0.56	>500	<b>&gt;99</b>
<b>Dexamethasone</b>	5.02 ± 1.34	159.2 ± 26.35	32

ND: Not determined due to insolubility in the media

## Molecular Docking of the $\beta$ -Lactams to the Human Nitric Oxide Synthase

Molecular docking studies were next performed to try to understand and further validate the anti-inflammatory activities, and to explore plausible binding interactions with the enzymatic pocket of the human inducible nitric oxide synthase protein. [41] The X-ray crystallographic structure of human inducible nitric oxide synthase (PDB ID: 4NOS) was selected as the docking template. The procedure was carried out by eliminating the structure of the co-crystallized ligand and redocking individual  $\beta$ -lactams (**3a-o**, **5a-h** or **6a-f**) into this region. Root-mean-square deviation (RMSD) of the Cartesian coordinates of the atoms of the ligand in the docked and crystallographic conformations is the principle of the docking validation ( $\text{RMSD} < 2 \text{ \AA}$ ). Validation of molecular docking showed RMSD value was for PDB ID: 4NOS =  $0.4 \text{ \AA}$ . [23] The scores of docking results based on the free binding energies and the hydrophobic and hydrogen bond interactions involved in the binding mode are presented in Table 4.

**Table 4.** Results of computational docking of morpholine-substituted  $\beta$ -lactams **3a-o**, **5a-h** and **6a-f** into the active site of human inducible nitric oxide synthase (PDB ID: 4NOS)

Code	$\Delta G_{\text{binding}}$ (Kcal/mol)	Hydrogen bond	Hydrophobic interaction	$\pi$ - $\pi$	Cation- $\pi$
<b>3a</b>	-8.97	Tyr373, Gln263	Asp382, Tyr347, Trp346, Val352, Pro350, Glu377, Ala262, Ala351, Asn354, Met355	Hem	-
<b>3b</b>	-9.74	-	Gly371, Val352, Ala262, Pro350, Ala351, Asn370, Glu377, Gln263, Tyr373, Asp382	-	-
<b>3c</b>	-10.20	-	Asp382, Tyr373, Glu377, Val352, Gln263, Met355, Ala262, Pro350, Ala351, Phe369, Asn354, Tyr491	-	-
<b>3d</b>	-8.77	Met480	Trp461, Glu479, Ile462, Cys115, Gly117, Cys110	-	Zn
<b>3e</b>	-9.34	Met480	Ser118, Asn482, Ile462, Cys110, Cys115, Gly117, Trp461, Glu479	-	Zn
<b>3f</b>	-9.04	Met480	Trp461, Glu479, Ile462, Asp460, Asn482, Cys115, Gly117, Cys110	-	Zn

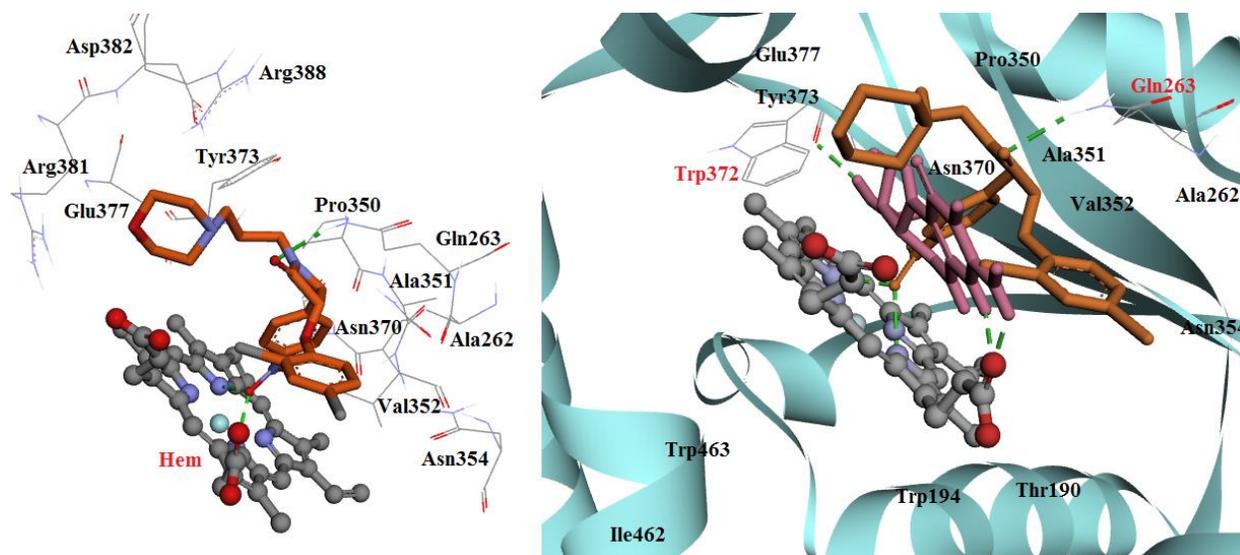
<b>3g</b>	-8.36	-	Ile462, Gly117, Cys115, Asp460, Asn482, Cys110, Ser118, Met480, Glu479, Trp461	-	Zn
<b>3h</b>	-8.35	-	Cys110, Trp461, Cys115, Ile462, Ser118, Gly117, Asp460, Asn482	-	Zn
<b>3i</b>	-10.34	-	Asp460, Asn482, Gly117, Ile462, Cys115, Ser118, Cys110, Met480, Phe476, Glu479, Trp461, Gln478	Trp461	Zn
<b>3j</b>	-7.49	-	Trp461, Ile462, Met480, Asn482, Cys115, Cys110, Gly117	-	Zn
<b>3k</b>	-8.16	Tyr347	Met355, Val352, Asn370, Gln263, Ala351, Glu377, Pro350, Tyr373, Asp382	Hem	-
<b>3l</b>	-8.48	Tyr373	Glu377, Asp382, Arg381, Gln263, Val352, Ala351, Pro350	-	-
<b>3m</b>	-9.56	Tyr373	Arg381, Asp382, Glu377, Gln263, Pro350, Ala351, Val352, Gly371, Asn370, Phe369	-	-
<b>3n</b>	-6.79	Gln263	Phe369, Gly371, Asn370, Ala351, Tyr373, Pro350, Val352, Glu377, Tyr347,	-	-
<b>3o</b>	-8.25	-	Ile462, Met480, Trp461, Asn482, Ala459, Asp460, Gly117, Ser118, Cys115	Trp461	-
<b>5a</b>	-9.85	-	Phe369, Asn354, Ala262, Ala351, Val352, Gln263, Pro350, Glu377, Tyr373, Asp382, Arg388, Arg381	-	-
<b>5b</b>	-10.62	-	Arg388, Asp382, Arg381, Ala351, Phe369, Glu377, Tyr373, Gln263, Ala262, Val352, Pro350	Hem	-
<b>5c</b>	-10.74	Gln263	Gly371, Asn370, Pro350, Val352, Tyr373, Ala351, Ala262, Arg388, Asn354, Glu377, Arg381, Asp382	-	-
<b>5d</b>	-8.04	Tyr373	Asn370, Ala351, Phe369, Pro350, Val352, Gly371, Met355, Ala262, Glu377,	Hem	-

			Gln263, Asp382, Arg381, Arg388		
<b>5e</b>	-9.55	Gln263	Asn370, Gly371, Phe369, Tyr373, Ala351, Pro350, Asp382, Arg388, Glu377, Arg381	-	-
<b>5f</b>	-10.58	Gln263	Arg388, Arg381, Asp382, Pro350, Glu377, Tyr373, Met355, Phe369, Val352, Asn354, Tyr491, Ala262, Asn370	-	-
<b>5g</b>	-8.44	Gln263	Arg388, Glu377, Asp382, Arg381, Ala262, Ala351, Val352, Phe369	-	-
<b>5h</b>	-8.23	-	Gly117, Cys110, Cys115, Trp461, Met480	-	Zn
<b>6a</b>	-8.51	Asp382, Asn354	Tyr373, Pro350, Gly371, Trp372, Glu377, Val352, Tyr491, Ala262	Hem	-
<b>6b</b>	-7.55	Gln263	Asp382, Arg381, Glu377, Arg388, Tyr373, Asn354, Tyr491, Val352, Ala351, Pro350	Hem	-
<b>6c</b>	-7.66	Tyr373	Ala351, Gln263, Pro350, Val352, Asp382, Glu377, Arg381, Trp463	-	-
<b>6d</b>	-8.38	Tyr373	Asn370, Gly371, Pro350, Ala351, Val352, Glu377, Asp382, Arg381	-	-
<b>6e</b>	-8.38	-	Pro350, Ala351, Asn370, Gln263, Val352, Gly371, Tyr347, Tyr373, Glu377, Asp382, Arg381, Arg388	-	-
<b>6f</b>	-9.25	Tyr373, Asn354	Asn370, Pro350, Gly371, Phe369, Val352, Glu377, Asp382, Gln263, Trp346, Tyr347, Tyr491, Ala262	-	-
<b>Dex</b>	-8.01	Tyr373, Arg388, Gln263, Glu377	Asp382, Val352, Phe369, Asn370, Pro350, Gly371	-	-

**Dex** = Dexamethasone

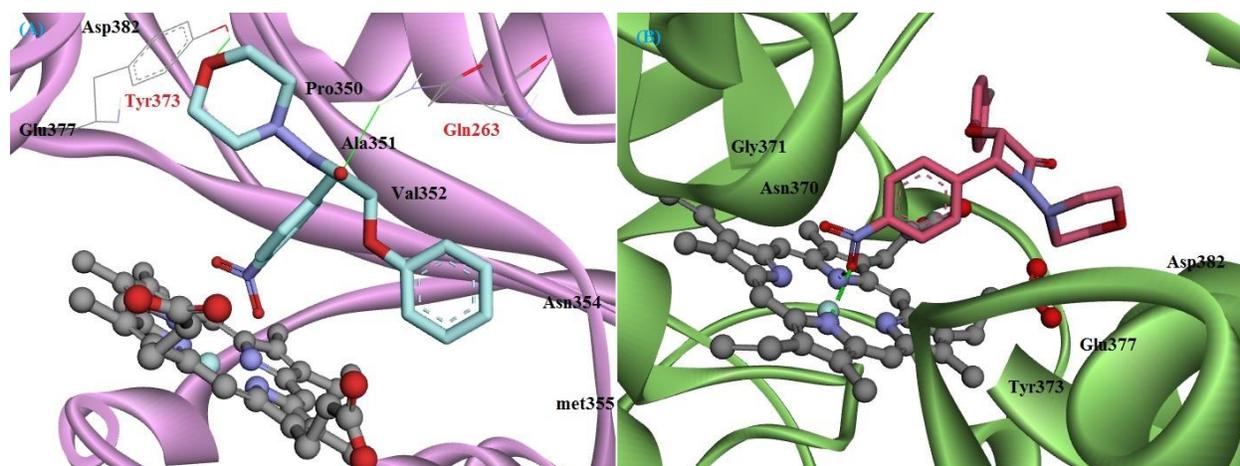
Compound **5c** exhibited the best docking score from these computational experiments, and displays the most potent *in vitro* anti-inflammatory activity after **3o**. Molecular docking of **5c** in human inducible nitric oxide synthase revealed an optimal binding mode characterized by the minimum Gibbs binding energy ( $\Delta G_{\text{bind}} = -10.74$  kcal/mol). As illustrated in the 3D diagram of **5c**

and in the superimposition of **5c** and co-crystallized ligand (Fig. 3), **5c** computationally docks almost at the same position as the original co-crystallized ligand. Looking next at the 2D image of the modeled **5c**-enzyme modeled (shown on the rightside of Fig. 3), the lactam carbonyl oxygen serves as a hydrogen bond acceptor with the Gln263 (highlighted in the yellow ball) while the other substituents of **5c** form hydrophobic interactions with residues Gly371, Asn370, Pro350, Val352, Tyr373, Ala351, Ala262, Arg388, Asn354, Glu377, Arg381 and Asp382 residues.



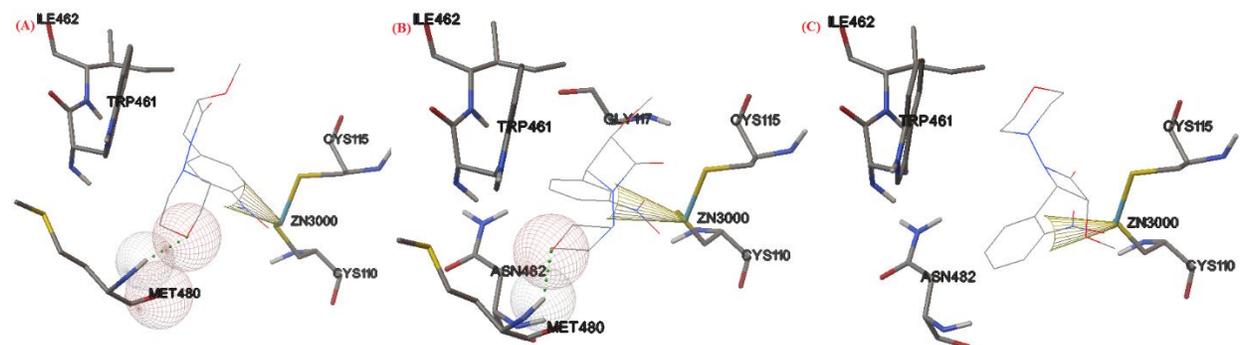
**Fig. 3.** Compound **5c** in the human inducible nitric oxide synthase active site (left) and superimposition of **5c** (brown) and co-crystallized ligand (purple) within binding site.

The presence of an aromatic ring at the C3 position of the  $\beta$ -lactam ring showed more potent anti-inflammatory activity than an aliphatic moiety at the same position. For example, compounds **3d**, **3f**, **3j** and **3n** having a methoxy group exhibited poor anti-inflammatory activity compared to **3a**, **3e**, **3g** and **3k** with a phenoxy group. Furthermore, replacing the phenyl ring (**3a**) with the *p*-chlorophenyl ring (**3b**) decreased both the anti-inflammatory activity and the nitric oxide synthase docking score. A comparison of the computed binding mode of **3a** and **3b** in the active site of the nitric oxide synthase is shown in Fig. 4. These images indicate that the active site binding modes of **3a** and **3b** are slightly different, such that a non-optimal orientation of **3b** might be the reason for the decrease in both the anti-inflammatory activity and the nitric oxide synthase docking score of **3b** compared to **3a**. Specifically, **3a** is computed to form a  $\pi$ - $\pi$  stacking interaction with Hem (Figure 4A), while **3b** lacks this contact (Figure 4B).



**Fig. 4.** Computational binding modes of compound **3a** (A) and **3b** (B) in the active site of human inducible nitric oxide synthase.

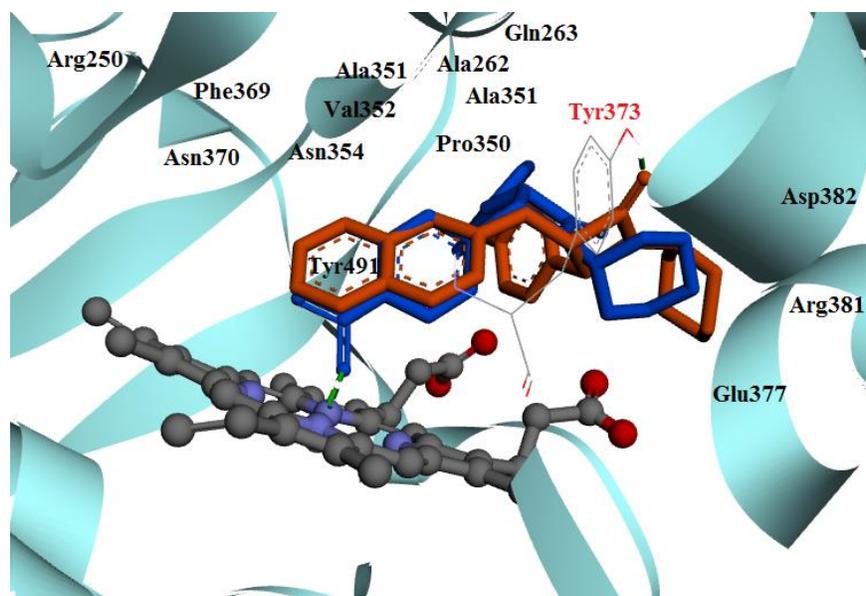
Similarly, nitrophenyl-substituted  $\beta$ -lactam analogues **3d**, **3f** and **3j** were computationally docked into the nitric oxide synthase binding site. Fig. 5 shows that the binding modes of **3d** and **3f** are similar but that of **3j** is different. The oxygen atom of the morpholine ring on the lactam nitrogen of **3d** and **3f** forms a tight hydrogen bond with the NH of Met480, but this stabilizing interaction is not observed for **3j**. We attribute this difference to be a plausible explanation for the diminished anti-inflammatory activity of **3j** to **3d** or **3f**.



**Fig. 5.** Superimposition of **3d** (A), **3f** (B) and **3j** (C) within the nitric oxide synthase binding site.

Furthermore, replacement of the nitrophenyl group of analogue **3c** with a cyanophenyl ring (**3m**) substantially decreased the *in vitro* NO inhibition activity, that is borne out in the *in silico* synthase

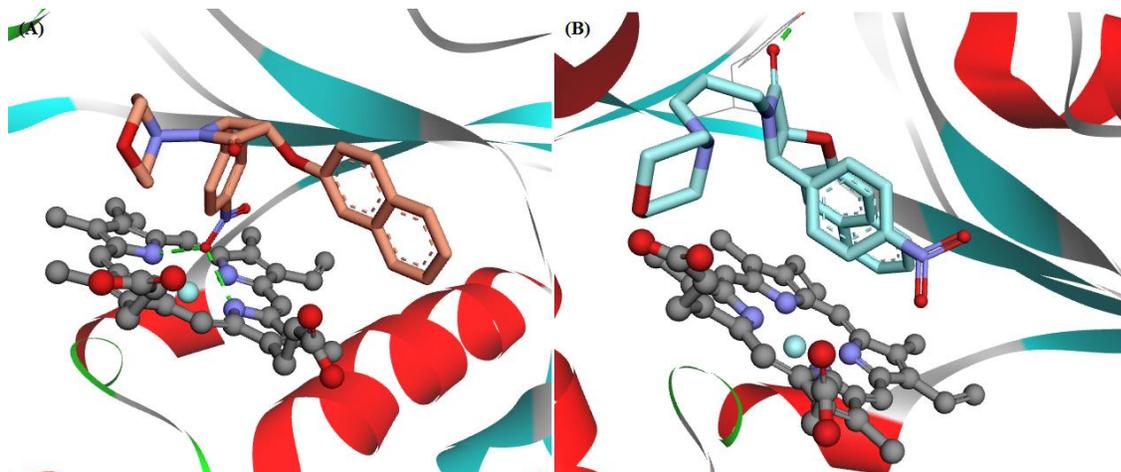
docking studies. The superimposition of compounds **3c** and **3m** are illustrated in Fig. 6, indicating that the nitrophenyl ring of **3c** interacts much more tightly with the porphyrin ring system of the synthase compared to the cyanophenyl ring of **3m**.



**Fig. 6.** Superimposition of **3c** (blue) with **3m** (orange) in binding site human inducible nitric oxide synthase enzyme.

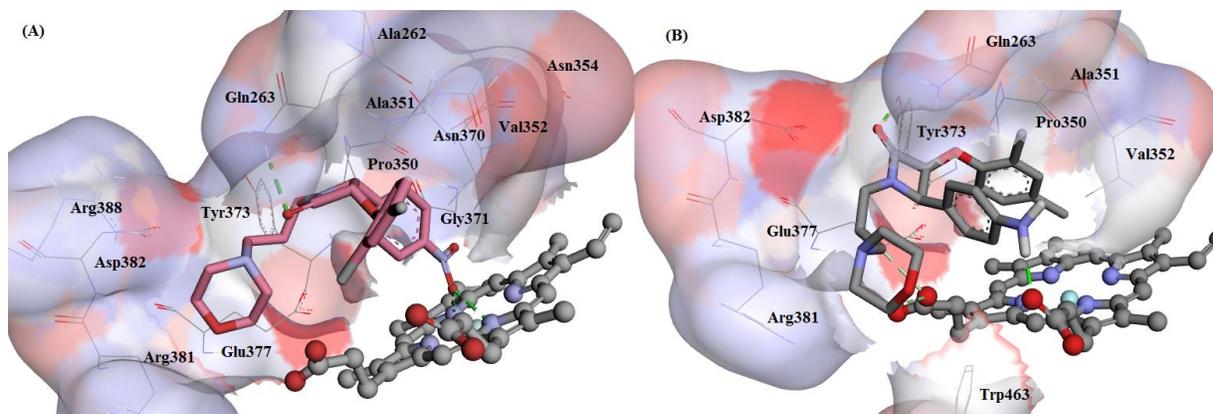
We next examined the difference between having the morpholine ring directly attached to the nitrogen of the  $\beta$ -lactam ring (analogue **3c**), versus having it tethered to the nitrogen via a three-carbon alkyl chain (analogue **5d**). *N*-Morpholine  $\beta$ -lactam **3c** has weaker *in vitro* anti-inflammatory activity than propylmorpholine  $\beta$ -lactam **5d** (Table 3). Computational modeling suggests that increased alkyl chain length has a negative effect on the active site docking ability (Fig. 7). The greater distance and conformational flexibility of the alkyl tether between the morpholine moiety and the  $\beta$ -lactam ring is predicted to reduce the effectiveness of reinforcing active site interactions. This should lower the NO inhibitory activity of **5d** versus **3c**. However, the opposite is observed. We believe that the sizable difference in lipophilic character of the two compounds may override the potential for stronger active site binding of **3c** over **5d**. The calculated CLogP of **3c** (CLogP = 2.32) is considerably lower than that of **5d** (CLogP = 4.67), which predicts

a much reduced capability of **3c** to penetrate the cell membrane, and this could be a reason for **3c** having lower anti-inflammatory potency than **5d**.



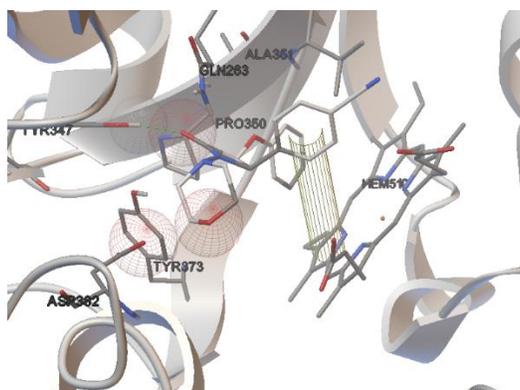
**Fig. 7.** Computational docking of **3c** (A) and **5d** (B) into the active site of human inducible nitric oxide synthase

Replacing the C4 nitrophenyl ring substituent (**5c**) with the corresponding C4 aminophenyl moiety (**6c**) simultaneously diminished the *in vitro* anti-inflammatory activity and the computational docking score (Table 3 and 4). This suggests that the NO<sub>2</sub> substituent may exert more favorable active site binding, as illustrated in the more fitted, flattened orientation of **5c** versus **6c** in the human nitric acid synthase active site (Fig. 8).



**Fig. 8.** Interactions of **5c** (A) and **6c** (B) in the active site of human inducible nitric oxide synthase

Additionally, placement of an additional chlorine substituent on the C3 phenyl ring of *para*-chlorophenyl derivatives **5b** and **6b** with a second chlorine at the *meta* position (**5c** and **6c**) further enhanced the *in vitro* anti-inflammatory activity and *in silico* enzyme binding affinity (Tables 3 and 4). This suggests that the presence of two chlorine atoms enhances opportunities for favorable hydrophobic interactions with active site residues, versus the mono-chlorophenyl substituent (ClogP = 3.91 for **6c** vs. ClogP = 3.38 for **6b**). In summary,  $\beta$ -lactams **6a-e** exhibited the weakest affinity to the substrate binding region of the NO synthase enzyme, compared to analogues **3a-o** and **5a-h**. Our molecular docking results suggest that compounds **3a**, **3i**, **3k**, **3o**, **5b**, **5d**, **6a**, and **6b** all form a favorable  $\pi$ - $\pi$  stacking interaction with Hem or Trp461 in the binding pocket (Fig. 9).



**Fig. 9.** Close-up view of the computational docking of **3k** within the active site of human inducible nitric oxide synthase.

## Materials

Chemicals were obtained from the Fluka, Merck, and Acros chemical companies.  $\text{CH}_2\text{Cl}_2$  and  $\text{Et}_3\text{N}$  were dried by distillation over  $\text{CaH}_2$  and then stored over 4  $\text{Å}$  molecular sieves.  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectra were recorded in  $\text{CDCl}_3$  or  $\text{DMSO-d}_6$  using a Bruker Avance DPX instrument (operating at 250 MHz for  $^1\text{H-NMR}$  and 62.9 MHz for  $^{13}\text{C-NMR}$ ). Chemical shifts were reported in ppm ( $\delta$ ) downfield from TMS. All the coupling constants ( $J$ ) are in Hertz. FTIR spectra were run on a Shimadzu FT-IR 8300 spectrophotometer. The mass spectra were recorded on a Shimadzu Mass spectra were obtained on a Shimadzu GCMS-QP 1000 EX instrument at 70 eV. Elemental analyses were run on a Thermo Finnigan Flash EA-1112 series. Melting points were

determined in open capillary tubes in Thermo Fisher scientific IA9200 apparatus and are uncorrected. The progress of the reactions was monitored by TLC on silica gel 254 analytical sheets obtained from Fluka and purification was achieved by silica gel column chromatography on Merck Kieselgel (230–270 mesh).

### General Procedure for the Synthesis of Monocyclic $\beta$ -Lactams (**3a-o**):

A solution of the corresponding Schiff base (**1**) (1.00 mmol) was stirred with the corresponding substituted acetic acid (**2**) (1.50 mmol), *p*-toluenesulfonyl chloride (1.50 mmol) and triethylamine (2.50 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> at room temperature. After 24 h, the mixture was washed with 1N aqueous HCl solution (20 mL), saturated aqueous NaHCO<sub>3</sub> solution (20 mL), brine (20 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and then filtered. After evaporation of solvent, the crude  $\beta$ -lactam products (**3a-o**) were purified by silica gel column chromatography (eluent 2:1 *n*-hexane/EtOAc).

#### *1-Morpholino-4-(4-nitrophenyl)-3-phenoxyazetid-2-one (3a)*

Cream-colored solid (yield 32 %). mp 164-166 °C. IR (KBr, cm<sup>-1</sup>) 1774 (CO,  $\beta$ -lactam), 1342, 1520 (NO<sub>2</sub>). <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  2.93 (CH<sub>2</sub>-N, 2H, m), 3.09 (CH<sub>2</sub>-N, 2H, m), 3.68 (CH<sub>2</sub>-O, 4H, m), 4.41 (H-4, d, *J* = 4.6 Hz, 1H), 5.35 (H-3, d, *J* = 4.6 Hz, 1H), 6.68 (ArH, d, *J* = 7.8 Hz, 2H), 6.86 (ArH, t, *J* = 7.3 Hz, 1H), 7.09 (ArH, t, *J* = 7.3 Hz, 2H), 7.63 (ArH, d, *J* = 8.6 Hz, 2H), 8.14 (ArH, d, *J* = 8.6 Hz, 2H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  53.5 (CH<sub>2</sub>-N), 66.2 (CH<sub>2</sub>-O), 62.3 (C-4), 79.3 (C-3), 115.2, 122.5, 123.4, 129.4, 129.8, 141.3, 148.2, 156.3 (aromatic carbons), 164.1 (CO,  $\beta$ -lactam). MS *m/z* = 369 [M<sup>+</sup>]. Anal. Calcd. for C<sub>19</sub>H<sub>19</sub>N<sub>3</sub>O<sub>5</sub>: C, 61.78; H, 5.18; N, 11.38 %. Found: C, 61.65; H, 5.29; N, 11.25 %.

#### *3-(4-Chlorophenoxy)-1-morpholino-4-(4-nitrophenyl) azetid-2-one (3b)*

White crystals (yield 35 %). mp 216-218 °C. IR (KBr, cm<sup>-1</sup>) 1786 (CO,  $\beta$ -lactam), 1346, 1520 (NO<sub>2</sub>). <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  2.91 (CH<sub>2</sub>-N, m, 2H), 3.07 (CH<sub>2</sub>-N, m, 2H), 3.65 (CH<sub>2</sub>-O, m, 4H), 5.24 (H-4, d, *J* = 4.9 Hz, 1H), 5.28 (H-3, d, *J* = 4.9 Hz, 1H), 6.65 (ArH, d, *J* = 9.0 Hz, 2H), 7.07 (ArH, d, *J* = 9.0 Hz, 2H), 7.62 (ArH, d, *J* = 8.8 Hz, 2H), 8.18 (ArH, d, *J* = 8.8 Hz, 2H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  53.5 (CH<sub>2</sub>-N), 62.2 (C-4), 66.2 (CH<sub>2</sub>-O), 79.4 (C-3), 116.6, 123.5, 127.6, 129.4,

129.8, 141.0, 148.3, 154.9 (aromatic carbons), 163.8 (CO,  $\beta$ -lactam). MS  $m/z$  = 403 [ $M^+$ ]. Anal. Calcd. for  $C_{19}H_{18}ClN_3O_5$ : C, 56.51; H, 4.49; N, 10.41 %. Found: C, 56.58; H, 4.61; N, 10.40 %.

*1-Morpholino-3-(naphthalen-2-yloxy)-4-(4-nitrophenyl) azetidin-2-one (3c)*

Cream-colored solid (yield 38 %). mp 236-238 °C. IR (KBr,  $cm^{-1}$ ) 1767 (CO,  $\beta$ -lactam), 1350, 1520 ( $NO_2$ ).  $^1H$ -NMR ( $CDCl_3$ )  $\delta$  2.86 ( $CH_2$ -N, m, 4H), 3.57 ( $CH_2$ -O, m, 4H), 5.26 (H-4, d,  $J$  = 4.6 Hz, 1H), 5.41 (H-3, d,  $J$  = 4.6 Hz, 1H), 6.79 (ArH, d,  $J$  = 8.9 Hz, 1H), 6.94 (ArH, s, 1H), 7.18-7.34 (ArH, m, 2H), 7.50 (ArH, d,  $J$  = 9.2 Hz, 2H), 7.58 (ArH, d,  $J$  = 8.8 Hz, 2H), 7.59 (ArH, d,  $J$  = 8.9 Hz, 1H), 8.04 (ArH, d,  $J$  = 8.8 Hz, 2H).  $^{13}C$ -NMR ( $CDCl_3$ )  $\delta$  53.5 ( $CH_2$ -N), 62.4 (C-4), 66.2 ( $CH_2$ -O), 79.2 (C-3), 108.7, 117.9, 123.4, 124.5, 126.7, 126.8, 127.7, 129.6, 129.8, 133.7, 141.2, 148.9, 157.3 (aromatic carbons), 163.9 (CO,  $\beta$ -lactam). MS  $m/z$  = 419 [ $M^+$ ]. Anal. Calcd. for  $C_{23}H_{21}N_3O_5$ : C, 65.86; H, 5.05; N, 10.02 %. Found: C, 66.01; H, 5.12; N, 9.89 %.

*3-Methoxy-1-morpholino-4-(4-nitrophenyl) azetidin-2-one (3d)*

White crystals (yield 45 %). mp 172-174 °C. IR (KBr,  $cm^{-1}$ ) 1782 (CO,  $\beta$ -lactam), 1350, 1520 ( $NO_2$ ).  $^1H$ -NMR ( $CDCl_3$ )  $\delta$  2.80 ( $CH_2$ -N, m, 4H), 3.08 (OMe, s, 3H), 3.60 ( $CH_2$ -O, m, 4H), 4.57 (H-4, d,  $J$  = 4.8 Hz, 1H), 5.06 (H-3, d,  $J$  = 4.8 Hz, 1H), 7.61 (ArH, d,  $J$  = 8.7 Hz, 2H), 8.19 (ArH, d,  $J$  = 8.7 Hz, 2H).  $^{13}C$ -NMR ( $CDCl_3$ )  $\delta$  53.4 ( $CH_2$ -N), 58.6 (OMe), 61.8 (C-4), 66.2 ( $CH_2$ -O), 83.1 (C-3), 123.4, 129.7, 142.0, 148.1 (aromatic carbons), 165.2 (CO,  $\beta$ -lactam). MS  $m/z$  = 307 [ $M^+$ ]. Anal. Calcd. for  $C_{14}H_{17}N_3O_5$ : C, 54.72; H, 5.58; N, 13.67 %. Found: C, 54.80; H, 5.67; N, 13.59 %.

*1-Morpholino-4-(3-nitrophenyl)-3-phenoxyazetidin-2-one (3e)*

Light brown solid (yield 25 %). mp 134-136 °C. IR (KBr,  $cm^{-1}$ ) 1770 (CO,  $\beta$ -lactam), 1350, 1527 ( $NO_2$ ).  $^1H$ -NMR ( $CDCl_3$ )  $\delta$  2.96 ( $CH_2$ -N, m, 2H), 3.10 ( $CH_2$ -N, m, 2H), 3.66 ( $CH_2$ -O, m, 4H), 5.27 (H-4, d,  $J$  = 4.7 Hz, 1H), 5.35 (H-3, d,  $J$  = 4.7 Hz, 1H), 6.67 (ArH, d,  $J$  = 8.5 Hz, 2H), 6.87 (ArH, t,  $J$  = 7.4 Hz, 1H), 7.08 (ArH, t,  $J$  = 8.5 Hz, 2H), 7.49 (ArH, t,  $J$  = 8.0 Hz, 1H), 7.80 (ArH, d,  $J$  = 8.0 Hz, 1H), 8.11 (ArH, d,  $J$  = 8.5 Hz, 1H), 8.31 (ArH, s, 1H).  $^{13}C$ -NMR ( $CDCl_3$ )  $\delta$  53.5 ( $CH_2$ -N), 62.3 (C-4), 66.2 ( $CH_2$ -O), 79.1 (C-3), 115.2, 122.4, 123.9, 123.9, 129.2, 129.4, 134.8, 136.2, 148.0, 156.2 (aromatic carbons), 164.1 (CO,  $\beta$ -lactam). MS  $m/z$  = 369 [ $M^+$ ]. Anal. Calcd. for  $C_{19}H_{19}N_3O_5$ : C, 61.78; H, 5.18; N, 11.38 %. Found: C, 61.69; H, 5.29; N, 11.35 %.

*3-Methoxy-1-morpholino-4-(3-nitrophenyl) azetid-2-one (3f)*

White crystals (yield 43%). mp 156-158 °C. IR (KBr,  $\text{cm}^{-1}$ ) 1782 (CO,  $\beta$ -lactam), 1342, 1535 ( $\text{NO}_2$ ).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  2.86 ( $\text{CH}_2\text{-N}$ , m, 4H), 3.14 (OMe, s, 3H), 3.63 ( $\text{CH}_2\text{-O}$ , m, 4H), 4.57 (H-4, d,  $J = 4.8$  Hz, 1H), 5.06 (H-3, d,  $J = 4.8$  Hz, 1H), 7.55 (ArH, t,  $J = 7.9$  Hz, 1H), 7.80 (ArH, d,  $J = 7.9$  Hz, 1H), 8.22 (ArH, d,  $J = 8.1$  Hz, 1H), 8.30 (ArH, s, 1H).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  53.5 ( $\text{CH}_2\text{-N}$ ), 58.6 (OMe), 61.9 (C-4), 66.2 ( $\text{CH}_2\text{-O}$ ), 82.8 (C-3), 123.8, 129.3, 134.7, 136.8, 148.2 (aromatic carbons), 165.3 (CO,  $\beta$ -lactam). MS  $m/z = 307$  [ $\text{M}^+$ ]. Anal. Calcd. for  $\text{C}_{14}\text{H}_{17}\text{N}_3\text{O}_5$ : C, 54.72; H, 5.58; N, 13.67 %. Found: C, 54.67; H, 5.65; N, 13.71 %.

*1-Morpholino-4-(2-nitrophenyl)-3-phenoxyazetid-2-one (3g)*

Cream-colored crystals (yield 36 %). mp 184-186 °C. IR (KBr,  $\text{cm}^{-1}$ ) 1767 (CO,  $\beta$ -lactam), 1354, 1527 ( $\text{NO}_2$ ).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  3.16 ( $\text{CH}_2\text{-N}$ , m, 4H), 3.66 ( $\text{CH}_2\text{-O}$ , m, 4H), 5.42 (H-4, d,  $J = 5.1$  Hz, 1H), 5.84 (H-3, d,  $J = 5.1$  Hz, 1H), 6.78 (ArH, d,  $J = 8.5$  Hz, 2H), 6.89 (ArH, t,  $J = 7.5$  Hz, 1H), 7.10 (ArH, t,  $J = 8.5$  Hz, 2H), 7.47 (ArH, t,  $J = 8.6$  Hz, 1H), 7.68 (ArH, t,  $J = 8.2$  Hz, 1H), 7.81 (ArH, d,  $J = 8.6$  Hz, 1H), 8.01 (ArH, d,  $J = 8.2$  Hz, 1H).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  53.2 ( $\text{CH}_2\text{-N}$ ), 59.5 (C-4), 66.5 ( $\text{CH}_2\text{-O}$ ), 80.2 (C-3), 115.8, 122.5, 124.9, 129.2, 129.4, 129.9, 130.5, 133.2, 148.8, 156.8 (aromatic carbons), 165.8 (CO,  $\beta$ -lactam). MS  $m/z = 369$  [ $\text{M}^+$ ]. Anal. Calcd. for  $\text{C}_{19}\text{H}_{19}\text{N}_3\text{O}_5$ : C, 61.78; H, 5.18; N, 11.38 %. Found: C, 61.85; H, 5.37; N, 11.42 %.

*3-(4-Chlorophenoxy)-1-morpholino-4-(2-nitrophenyl) azetid-2-one (3h)*

Cream-colored crystals (yield 23 %). mp 152-154 °C. IR (KBr,  $\text{cm}^{-1}$ ) 1782 (CO,  $\beta$ -lactam), 1342, 1527 ( $\text{NO}_2$ ).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  3.21 ( $\text{CH}_2\text{-N}$ , m, 4H), 3.72 ( $\text{CH}_2\text{-O}$ , m, 4H), 5.36 (H-4, d,  $J = 5.0$  Hz, 1H), 5.81 (H-3, d,  $J = 5.0$  Hz, 1H), 6.75 (ArH, d,  $J = 8.6$  Hz, 2H), 7.09 (ArH, d,  $J = 8.6$  Hz, 2H), 7.49 (ArH, t,  $J = 7.5$  Hz, 1H), 7.68 (ArH, t,  $J = 7.5$  Hz, 1H), 7.80 (ArH, d,  $J = 7.6$  Hz, 1H), 8.00 (ArH, d,  $J = 8.1$  Hz, 1H).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  53.2 ( $\text{CH}_2\text{-N}$ ), 59.6 (C-4), 66.5 ( $\text{CH}_2\text{-O}$ ), 80.4 (C-3), 117.3, 125.1, 128.8, 129.3, 129.7, 130.3, 130.9, 133.3, 148.7, 155.4 (aromatic carbons), 165.5 (CO,  $\beta$ -lactam). MS  $m/z = 403$  [ $\text{M}^+$ ]. Anal. Calcd. for  $\text{C}_{19}\text{H}_{18}\text{ClN}_3\text{O}_5$ : C, 56.51; H, 4.49; N, 10.41 %. Found: C, 56.60; H, 4.62; N, 10.36 %.

*1-Morpholino-3-(naphthalen-2-yloxy)-4-(2-nitrophenyl) azetidin-2-one (3i)*

Cream-colored solid (yield 38 %); mp 202-204 °C; IR (KBr, cm<sup>-1</sup>) 1767 (CO,  $\beta$ -lactam), 1342, 1527 (NO<sub>2</sub>). <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  3.16 (CH<sub>2</sub>-N, m, 4H), 3.72 (CH<sub>2</sub>-O, m, 4H), 5.57 (H-4, d,  $J$  = 5.1 Hz, 1H), 5.92 (H-3, d,  $J$  = 5.1 Hz, 1H), 6.87 (ArH, d,  $J$  = 8.9 Hz, 1H), 7.26 (ArH, s, 1H), 7.31–7.45 (ArH, m, 3H), 7.57 (ArH, d,  $J$  = 8.9 Hz, 2H), 7.66 (ArH, d,  $J$  = 9.1 Hz, 2H), 7.85 (ArH, d,  $J$  = 7.9 Hz, 1H), 7.96 (ArH, d,  $J$  = 8.2 Hz, 1H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  53.3 (CH<sub>2</sub>-N), 59.3 (C-4), 66.5 (CH<sub>2</sub>-O), 80.2 (C-3), 109.6, 118.1, 124.4, 124.9, 126.5, 127.1, 127.5, 129.3, 129.5, 129.6, 129.9, 130.1, 133.1, 133.9, 148.9, 154.5 (aromatic carbons), 165.5 (CO,  $\beta$ -lactam). MS  $m/z$  = 419 [M<sup>+</sup>]. Anal. Calcd. for C<sub>23</sub>H<sub>21</sub>N<sub>3</sub>O<sub>5</sub>: C, 65.86; H, 5.05; N, 10.02 %. Found: C, 65.90; H, 5.17; N, 10.00 %.

*3-Methoxy-1-morpholino-4-(2-nitrophenyl) azetidin-2-one (3j)*

White solid (yield 64 %). mp 180-182 °C; IR (KBr, cm<sup>-1</sup>) 1767 (CO,  $\beta$ -lactam), 1342, 1520 (NO<sub>2</sub>). <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  3.08 (CH<sub>2</sub>-N, m, 4H), 3.21 (OMe, s, 3H), 3.61 (CH<sub>2</sub>-O, m, 4H), 4.68 (H-4, d,  $J$  = 5.0 Hz, 1H), 5.51 (H-3, d,  $J$  = 5.0 Hz, 1H), 7.47 (ArH, t,  $J$  = 8.7 Hz, 1H), 7.65 (ArH, t,  $J$  = 7.9 Hz, 1H), 7.72 (ArH, d,  $J$  = 7.9 Hz, 1H), 8.03 (ArH, d,  $J$  = 8.1 Hz, 1H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  53.2 (CH<sub>2</sub>-N), 59.0 (OMe), 59.8 (C-4), 66.5 (CH<sub>2</sub>-O), 83.6 (C-3), 124.9, 128.9, 129.9, 130.9, 133.2, 148.8 (aromatic carbons), 166.9 (CO,  $\beta$ -lactam). MS  $m/z$  = 307 [M<sup>+</sup>]; Anal. Calcd. for C<sub>14</sub>H<sub>17</sub>N<sub>3</sub>O<sub>5</sub>: C, 54.72; H, 5.58; N, 13.67%. Found: C, 54.91; H, 5.57; N, 13.79 %.

*4-(1-Morpholino-4-oxo-3-phenoxyazetidin-2-yl)benzotrile (3k)*

White crystals (yield 35 %). mp: 198-200 °C. IR (KBr, cm<sup>-1</sup>): 1767 (CO,  $\beta$ -lactam), 2229 (CN). <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  2.91 (CH<sub>2</sub>-N, m, 2H), 3.06 (CH<sub>2</sub>-N, m, 2H), 3.66 (4H, m, CH<sub>2</sub>-O), 5.20 (H-4, d,  $J$  = 4.7 Hz, 1H), 5.32 (H-3, d,  $J$  = 4.7 Hz, 1H), 6.66 (ArH, d,  $J$  = 8.6 Hz, 2H), 6.87 (ArH, t,  $J$  = 7.2 Hz, 1H), 7.09 (ArH, t,  $J$  = 8.6 Hz, 2H), 7.55-7.62 (ArH, m, 4H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  53.5 (CH<sub>2</sub>-N), 62.6 (C-4), 66.2 (CH<sub>2</sub>-O), 79.2 (C-3), 112.8, 115.2, 118.3, 122.5, 129.4, 129.6, 131.9, 139.3, 153.3 (aromatic carbons and CN), 164.1 (CO,  $\beta$ -lactam). MS  $m/z$  = 349 [M<sup>+</sup>]. Anal. Calcd. for C<sub>20</sub>H<sub>19</sub>N<sub>3</sub>O<sub>3</sub>: C, 68.75; H, 5.48; N, 12.03 %. Found: C, 68.70; H, 5.57; N, 11.99 %.

*4-(3-(4-Chlorophenoxy)-1-morpholino-4-oxoazetidin-2-yl)benzo nitrile (3l)*

White crystals (yield 29 %). mp 162-168 °C. IR (KBr, cm<sup>-1</sup>) 1789 (CO,  $\beta$ -lactam), 2229 (CN). <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  2.90 (CH<sub>2</sub>-N, m, 2H), 3.06 (CH<sub>2</sub>-N, m, 2H), 3.62 (CH<sub>2</sub>-O, m, 4H), 5.20 (H-4, d,  $J$  = 4.8 Hz, 1H), 5.28 (H-3, d,  $J$  = 4.8 Hz, 1H), 6.66 (ArH, d,  $J$  = 9.0 Hz, 2H), 7.07 (ArH, d,  $J$  = 9.0 Hz, 2H), 7.55 (ArH, d,  $J$  = 8.6 Hz, 2H), 7.61 (ArH, d,  $J$  = 8.6 Hz, 2H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  53.5 (CH<sub>2</sub>-N), 62.4 (C-4), 66.2 (CH<sub>2</sub>-O), 79.3 (C-3), 112.9, 116.6, 118.2, 127.6, 129.4, 129.6, 132.1, 139.0, 154.9 (aromatic carbons and CN), 163.8 (CO,  $\beta$ -lactam). MS m/z = 383 [M<sup>+</sup>]. Anal. Calcd. for C<sub>20</sub>H<sub>18</sub>ClN<sub>3</sub>O<sub>3</sub>: C, 62.58; H, 4.73; N, 10.95 %. Found: C, 62.59; H, 4.86; N, 10.92 %.

*4-(1-Morpholino-3-(naphthalene-2-yloxy)-4-oxoazetidin-2-yl) benzonitrile (3m)*

White solid (yield 49 %). mp 182-184 °C. IR (KBr, cm<sup>-1</sup>) 1774(CO,  $\beta$ -lactam), 2229 (CN). <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  2.92 (CH<sub>2</sub>-N, m, 4H), 3.67 (CH<sub>2</sub>-O, m, 4H), 5.28 (H-4, d,  $J$  = 4.7 Hz, 1H), 5.47 (H-3, d,  $J$  = 4.7 Hz, 1H), 6.85 (ArH, d,  $J$  = 8.9 Hz, 1H), 6.99 (ArH, s, 1H), 7.25-7.71 (ArH, m, 9H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  53.5 (CH<sub>2</sub>-N), 62.6 (C-4), 66.2 (CH<sub>2</sub>-O), 79.2 (C-3), 108.7, 112.8, 117.9, 118.3, 124.5, 126.7, 126.8, 127.7, 129.6, 129.8, 131.9, 133.8, 139.3, 154.2 (aromatic carbons and CN), 164.0 (CO,  $\beta$ -lactam). MS m/z = 399 [M<sup>+</sup>]. Anal. Calcd. for C<sub>24</sub>H<sub>21</sub>N<sub>3</sub>O<sub>3</sub>: C, 72.16; H, 5.30; N, 10.52 %. Found: C, 72.29; H, 5.57; N, 10.59 %.

*4-(3-Methoxy-1-morpholino-4-oxoazetidin-2-yl) benzonitrile (3n)*

White crystals (yield 36 %). mp: 136-138 °C. IR (KBr, cm<sup>-1</sup>): 1767 (CO,  $\beta$ -lactam), 2230 (CN). <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  2.86 (CH<sub>2</sub>-N, m, 4H), 3.11 (OMe, s, 3H), 3.64. (CH<sub>2</sub>-O, m, 4H), 4.56 (H-4, d,  $J$  = 4.7 Hz, 1H), 5.03 (H-3, d,  $J$  = 4.7 Hz, 1H), 7.59 (ArH, d,  $J$  = 8.2 Hz, 2H), 7.70 (ArH, d,  $J$  = 8.2 Hz, 2H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  53.4 (CH<sub>2</sub>-N), 58.5 (OMe), 62.1 (C-4), 66.2 (CH<sub>2</sub>-O), 83.0 (C-3), 112.6, 118.4, 129.5, 132.1, 140.0 (aromatic carbons and CN), 165.3 (CO,  $\beta$ -lactam). MS m/z = 287 [M<sup>+</sup>]. Anal. Calcd. for C<sub>15</sub>H<sub>17</sub>N<sub>3</sub>O<sub>3</sub>: C, 62.71; H, 5.96; N, 14.63 %. Found: C, 62.79; H, 6.07; N, 14.69 %.

*1-Morpholino-2-(2-nitrophenyl)spiro[azetidine-3,9'-xanthen]4-one (3o)*

Light yellow solid (yield 41 %). mp 198-200 °C. IR (KBr, cm<sup>-1</sup>) 1759 (CO,  $\beta$ -lactam), 1346, 1523 (NO<sub>2</sub>). <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  3.52-3.76 (CH<sub>2</sub>, m, 8H), 5.38 (H-4, s, 1H), 6.62-8.10 (ArH, m, 12H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  53.8 (CH<sub>2</sub>-N), 61.4 (C-3), 66.8 (CH<sub>2</sub>-O), 73.9 (C-4), 114.9, 116.8,

116.9, 120.5, 122.2, 123.9, 124.8, 125.1, 127.8, 128.9, 129.3, 129.5, 131.1, 133.1, 147.5, 152.1, 152.3 (aromatic carbons), 169.7 (CO,  $\beta$ -lactam). Anal. Calcd. for C<sub>25</sub>H<sub>21</sub>N<sub>3</sub>O<sub>5</sub>: C, 67.71; H, 4.77; N, 9.48 %. Found: C, 67.90; H, 4.56; N, 9.45 %.

### General procedure for the synthesis of $\beta$ -lactams **5a–h**

Morpholine- $\beta$ -lactam hybrid compounds **5a–h** have been synthesized according to the introduced route in Scheme 3. A mixture of the corresponding Schiff base (**4**) (5.00 mmol), triethylamine (25.00 mmol), acetic acid derivative (**2**) (7.5 mmol) and *p*-toluenesulfonyl chloride (7.5 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (25 mL) was stirred at room temperature overnight. After the reaction completion (TLC monitoring), the mixture was washed with HCl (1 N), saturated sodium bicarbonate solution, brine, dried (Na<sub>2</sub>SO<sub>4</sub>) and the solvent was evaporated to afford the crude  $\beta$ -lactams **5a–h** which were purified by gel column chromatography (eluent 10:1 EtOAc/EtOH).

#### *1-(3-Morpholinopropyl)-4-(4-nitrophenyl)-3-phenoxyazetid-2-one (5a)*

Off-white crystalline solid (yield 76 %). mp 153-155 °C. IR (KBr, cm<sup>-1</sup>) 1751 (CO,  $\beta$ -lactam), 1350, 1520 (NO<sub>2</sub>). <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  1.65 (aliphatic chain, m, 2H), 2.31 (aliphatic chain and morpholine ring, m, 6H), 2.97 (aliphatic chain, m, 1H), 3.52 (aliphatic chain and morpholine ring, m, 5H), 5.05 (H-4, d, *J* = 4.5 Hz, 1H), 5.48 (H-3, d, *J* = 4.5 Hz, 1H), 6.67 (ArH, d, *J* = 8.7 Hz, 2H), 6.83 (ArH, t, *J* = 7.7 Hz, 1H), 7.07 (ArH, t, *J* = 7.7 Hz, 2H), 7.48 (ArH, d, *J* = 8.7 Hz, 2H), 8.11 (ArH, d, *J* = 8.7 Hz, 2H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  24.4 (aliphatic chain), 39.2 (aliphatic chain), 53.6 (morpholine ring), 56.0 (aliphatic chain), 61.5 (C-4), 66.8 (morpholine ring), 81.9 (C-3), 115.2, 122.4, 123.4, 129.3, 129.4, 141.0, 148.1, 156.4 (aromatic carbons), 165.5 (CO,  $\beta$ -lactam). MS *m/z* = 411[M<sup>+</sup>]. Anal. Calcd. for C<sub>22</sub>H<sub>25</sub>N<sub>3</sub>O<sub>5</sub>: C, 64.22; H, 6.12; N, 10.21%. Found: C, 64.40; H, 6.25; N, 10.17 %.

#### *3-(4-Chlorophenoxy)-1-(3-morpholinopropyl)-4-(4-nitrophenyl) azetid-2-one (5b)*

Pea green solid (yield 74 %). mp 108-110 °C. IR (KBr, cm<sup>-1</sup>) 1751 (CO,  $\beta$ -lactam), 1350, 1520 (NO<sub>2</sub>). <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  1.68 (aliphatic chain, m, 2H), 2.31 (aliphatic chain and morpholine ring, m, 6H), 3.00 (aliphatic chain, m, 1H), 3.54 (aliphatic chain and morpholine ring, m, 5H), 5.04 (H-4, d, *J* = 4.5 Hz, 1H), 5.42 (H-3, d, *J* = 4.5 Hz, 1H), 6.66 (ArH, d, *J* = 8.6 Hz, 2H), 7.07 (ArH, d, *J* = 8.5 Hz, 2H), 7.49 (ArH, d, *J* = 8.6 Hz, 2H), 8.16 (ArH, d, *J* = 8.5 Hz, 2H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  24.4 (aliphatic chain), 39.2 (aliphatic chain), 53.6 (morpholine ring), 56.0

(aliphatic chain), 61.3 (C-4), 66.8 (morpholine ring), 82.0 (C-3), 116.6, 123.6, 127.5, 129.3, 129.4, 140.7, 148.2, 155.0 (aromatic carbons), 165.2 (CO,  $\beta$ -lactam). MS  $m/z = 443 [M^+]$ . Anal. Calcd. for  $C_{22}H_{24}ClN_3O_5$ : C, 59.26; H, 5.43; N, 9.42%. Found: C, 59.32; H, 5.49; N, 9.39 %.

*3-(2,4-Dichlorophenoxy)-1-(3-morpholinopropyl)-4-(4-nitro phenyl)azetidin-2-one (5c)*

White solid (yield 63 %). mp 114-116 °C. IR (KBr,  $cm^{-1}$ ) 1743 (CO,  $\beta$ -lactam), 1350, 1520 ( $NO_2$ ).  $^1H$ -NMR ( $CDCl_3$ )  $\delta$  1.62 (aliphatic chain, m, 2H), 2.25 (aliphatic chain and morpholine ring, m, 6H), 2.97 (aliphatic chain, m, 1H), 3.50 (aliphatic chain and morpholine ring, m, 5H), 5.02 (H-4, d,  $J = 4.8$  Hz, 1H), 5.37 (H-3, d,  $J = 4.8$  Hz, 1H), 6.96 (ArH, d,  $J = 8.7$  Hz, 1H), 7.02 (ArH, d,  $J = 8.7$  Hz, 1H), 7.13 (ArH, s, 1H), 7.48 (ArH, d,  $J = 8.7$  Hz, 2H), 8.15 (ArH, d,  $J = 8.7$  Hz, 2H).  $^{13}C$ -NMR ( $CDCl_3$ )  $\delta$  24.4 (aliphatic chain), 39.3 (aliphatic chain), 53.6 (morpholine ring), 56.0 (aliphatic chain), 60.9 (C-4), 66.8 (morpholine ring), 82.5 (C-3), 116.4, 123.6, 123.9, 127.6, 127.8, 129.4, 130.1, 140.6, 148.3, 151.2 (aromatic carbons), 164.9 (CO,  $\beta$ -lactam). MS  $m/z = 480 [M^+]$ . Anal. Calcd. for  $C_{22}H_{23}Cl_2N_3O_5$ : C, 55.01; H, 4.83; N, 8.75%. Found: C, 55.15; H, 4.97; N, 8.71 %.

*1-(3-Morpholinopropyl)-3-(naphthalen-2-yloxy)-4-(4-nitro phenyl)azetidin-2-one (5d)*

Pea green solid (yield 79 %). mp 133-135 °C. IR (KBr,  $cm^{-1}$ ) 1751 (CO,  $\beta$ -lactam), 1350, 1520 ( $NO_2$ ).  $^1H$ -NMR ( $CDCl_3$ )  $\delta$  1.69 (aliphatic chain, m, 2H), 2.29 (aliphatic chain and morpholine ring, m, 6H), 3.00 (aliphatic chain, 1H, m), 3.57 (aliphatic chain and morpholine ring, 5H, m), 5.15 (H-4, d,  $J = 4.4$  Hz, 1H), 5.64 (H-3, d,  $J = 4.4$  Hz, 1H), 6.84 (ArH, d,  $J = 8.9$  Hz, 1H), 7.06 (ArH, s, 1H), 6.26-7.69 (ArH, m, 7H), 8.10 (ArH, d,  $J = 8.7$  Hz, 2H).  $^{13}C$ -NMR ( $CDCl_3$ )  $\delta$  24.5 (aliphatic chain), 39.2 (aliphatic chain), 53.6 (morpholine ring), 56.0 (aliphatic chain), 61.5 (C-4), 66.8 (morpholine ring), 81.9 (C-3), 108.7, 118.0, 123.5, 124.4, 126.6, 126.8, 127.7, 129.3, 129.6, 129.7, 133.8, 140.9, 148.1, 154.3 (aromatic carbons), 165.4 (CO,  $\beta$ -lactam). MS  $m/z = 461 [M^+]$ ; Anal. Calcd. for  $C_{26}H_{27}N_3O_5$ : C, 67.66; H, 5.90; N, 9.10 %. Found: C, 67.54; H, 6.02; N, 9.13 %.

*1-(3-Morpholinopropyl)-4-(3-nitrophenyl)-3-phenoxyazetidin-2-one (5e)*

White solid (yield 69 %). mp 101-103 °C. IR (KBr,  $cm^{-1}$ ) 1751 (CO,  $\beta$ -lactam), 1350, 1527 ( $NO_2$ ).  $^1H$ -NMR ( $CDCl_3$ )  $\delta$  1.64 (aliphatic chain, m, 2H), 2.28 (aliphatic chain and morpholine

ring, m, 6H), 2.97 (aliphatic chain, m, 1H), 3.51 (aliphatic chain and morpholine ring, m, 5H), 5.00 (H-4, d,  $J = 4.4$  Hz, 1H), 5.43 (H-3, d,  $J = 4.4$  Hz, 1H), 6.62 (ArH, d,  $J = 7.9$  Hz, 2H), 6.81 (ArH, t,  $J = 7.5$  Hz, 1H), 7.02 (ArH, t,  $J = 7.9$  Hz, 2H), 7.40 (ArH, t, 1H), 7.62 (ArH, d,  $J = 7.8$  Hz, 1H), 8.05 (ArH, d,  $J = 8.1$  Hz, 1H), 8.12 (ArH, s, 1H).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  24.4 (aliphatic chain), 39.1 (aliphatic chain), 53.6 (morpholine ring), 56.0 (aliphatic chain), 61.5 (C-4), 66.7 (morpholine ring), 81.7 (C-3), 115.2, 122.4, 123.5, 123.8, 129.3, 129.4, 134.3, 135.8, 148.1, 156.3 (aromatic carbons), 165.5 (CO,  $\beta$ -lactam). MS  $m/z = 411[\text{M}^+]$ . Anal. Calcd. for  $\text{C}_{22}\text{H}_{25}\text{N}_3\text{O}_5$ : C, 64.22; H, 6.12; N, 10.21%. Found: C, 64.37; H, 6.20; N, 10.16 %.

*1-(3-Morpholinopropyl)-3-(naphthalen-2-yloxy)-4-(3-nitro phenyl) azetidin-2-one (5f)*

Off white crystals (yield 63 %). mp: 126-128 °C. IR (KBr,  $\text{cm}^{-1}$ ): 1759 (CO,  $\beta$ -lactam), 1350, 1527 ( $\text{NO}_2$ ).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  6.64 (H-3, d,  $J = 4.4$  Hz, 1H), 6.88 (ArH, d,  $J = 8.9$  Hz, 1H), 7.07 (ArH, s, 1H), 7.31-8.24 (ArH, m, 7H), 8.23 (ArH, d,  $J = 8.7$  Hz, 1H), 8.57 (ArH, s, 1H).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  24.4 (aliphatic chain), 39.2 (aliphatic chain), 53.5 (morpholine ring), 56.0 (aliphatic chain), 61.4 (C-4), 66.7 (morpholine ring), 81.7 (C-3), 108.8, 117.9, 123.4, 123.8, 124.4, 126.6, 126., 127.6, 129.3, 129.5, 129.7, 133.8, 134.4, 135.8, 148.1, 154.2 (aromatic carbons), 165.5 (CO,  $\beta$ -lactam). MS  $m/z = 461 [\text{M}^+]$ . Anal. Calcd. for  $\text{C}_{26}\text{H}_{27}\text{N}_3\text{O}_5$ : C, 67.66; H, 5.90; N, 9.10 %. Found: C, 67.74; H, 6.02; N, 9.13 %.

*1-(3-Morpholinopropyl)-2-(4-nitrophenyl)spiro[azetidine-3,9'-xanthen]-4-one (5g)*

White solid (yield 71 %). mp 160-162 °C. IR (KBr,  $\text{cm}^{-1}$ ) 1743 (CO,  $\beta$ -lactam), 1350, 1527 ( $\text{NO}_2$ ).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  1.93 (aliphatic chain, m, 2H), 2.44 (aliphatic chain and morpholine ring, 6H, m), 3.17 (aliphatic chain, m, 1H), 3.66 (morpholine ring, m, 4H), 3.96 (aliphatic chain, 1H, m), 4.73 (H-4, s, 1H), 6.82-7.43 (ArH, 10H, m), 7.95 (ArH, d,  $J = 8.7$  Hz, 2H).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  25.0 (aliphatic chain), 39.9 (aliphatic chain), 53.6 (morpholine ring), 56.0 (aliphatic chain), 65.4 (C-3), 66.8 (morpholine ring), 73.7 (C-4), 116.5, 116.7, 117.1, 121.2, 122.9, 123.4, 124.4, 125.1, 127.3, 127.7, 129.4, 129.5, 142.2, 147.3, 151.9, 152.1 (aromatic carbons), 168.0 (CO,  $\beta$ -lactam). MS  $m/z = 485 [\text{M}^+]$ ; Anal. Calcd. for  $\text{C}_{28}\text{H}_{27}\text{N}_3\text{O}_5$ : C, 69.26; H, 5.61; N, 8.65%. Found: C, 69.37; H, 5.74; N, 8.67 %.

*1-(3-Morpholinopropyl)-2-(3-nitrophenyl)spiro[azetidine-3,9'-xanthen]-4-one (5h)*

Light yellow crystalline solid (yield 59 %). mp 151-153 °C. IR (KBr, cm<sup>-1</sup>) 1751 (CO,  $\beta$ -lactam), 1342, 1527 (NO<sub>2</sub>). <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  1.94 (aliphatic chain, m, 2H), 2.49 (aliphatic chain and morpholine ring, m, 6H), 3.18 (aliphatic chain, m, 1H), 3.68 (morpholine ring, m, 4H), 3.94 (aliphatic chain, m, 1H), 4.72 (H-4, s, 1H), 6.79-7.94 (ArH, m, 12H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  24.9 (aliphatic chain), 39.9 (aliphatic chain), 53.5 (morpholine ring), 56.0 (aliphatic chain), 65.2 (C-3), 66.6 (morpholine ring), 73.4 (C-4), 116.4, 116.8, 117.1, 121.2, 121.3, 122.9, 123.0, 124.4, 125.1, 127.8, 129.3, 129.4, 129.4, 132.6, 136.9, 147.9, 151.9, 152.1 (aromatic carbons), 168.1 (CO,  $\beta$ -lactam). MS m/z = 485 [M<sup>+</sup>]. Anal. Calcd. for C<sub>28</sub>H<sub>27</sub>N<sub>3</sub>O<sub>5</sub>: C, 69.26; H, 5.61; N, 8.65%. Found: C, 69.30; H, 5.69; N, 8.67 %.

**General Procedure for the Synthesis of Monocyclic  $\beta$ -Lactams 6a–f:**

$\beta$ -Lactams **5a–f** (1 mmol) were dissolved in 80 mL of EtOH: H<sub>2</sub>O (9:1) by heating to reflux. Then the temperature was reduced to 60 °C and an 80% aqueous solution of hydrazine hydrate (0.15 mL, 2.3 mmol) and Raney-Ni (1 g as aqueous slurry) were added. The mixture was refluxed for an appropriate time for complete conversion by TLC monitoring. The mixture was cooled to room temperature and then filtered through a pad of Celite. The solvent was evaporated under reduced pressure and purified by silica gel chromatography (eluent 5:1 EtOAc/EtOH) to yield pure  $\beta$ -lactams **6a–f**.

*4-(4-Aminophenyl)-1-(3-morpholinopropyl)-3-phenoxyazetidin-2-one (6a)*

White solid (yield 96 %). mp 136-138 °C. IR (KBr, cm<sup>-1</sup>) 1751 (CO,  $\beta$ -lactam), 3356, 3456 (NH<sub>2</sub>). <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  1.62 (aliphatic chain, m, 2H), 2.29 (aliphatic chain and morpholine ring, m, 6H), 2.95 (aliphatic chain, m, 1H), 3.43 (aliphatic chain, m, 1H), 3.65 (morpholine ring and NH<sub>2</sub>, m, 6H), 4.81 (H-4, d,  $J$  = 4.3 Hz, 1H), 5.34 (H-3, d,  $J$  = 4.3 Hz, 1H), 6.55 (ArH, d,  $J$  = 8.4 Hz, 2H), 6.72 (ArH, d,  $J$  = 8.4 Hz, 2H), 6.83 (ArH, t,  $J$  = 7.3 Hz, 1H), 7.08 (ArH, d,  $J$  = 8.3 Hz, 2H), 7.14 (ArH, t,  $J$  = 8.6 Hz, 2H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  24.4 (aliphatic chain), 38.4 (aliphatic chain), 53.6 (morpholine ring), 56.2 (aliphatic chain), 62.2 (C-4), 66.9 (morpholine ring), 81.9 (C-3), 114.7, 115.6, 121.8, 122.2, 129.1, 129.8, 146.9, 157.0 (aromatic carbons), 166.1 (CO,  $\beta$ -lactam). MS m/z = 382 [M<sup>+</sup>]. Anal. Calcd. for C<sub>22</sub>H<sub>27</sub>N<sub>3</sub>O<sub>3</sub>: C, 69.27; H, 7.13; N, 11.02 %. Found: C, 69.35; H, 7.15; N, 10.95 %.

*4-(4-Aminophenyl)-3-(4-chlorophenoxy)-1-(3-morpholinopropyl) azetidín-2-one (6b)*

White solid (yield 92 %). mp 177-180 °C. IR (KBr, cm<sup>-1</sup>) 1735 (CO,  $\beta$ -lactam), 3332, 3409 (NH<sub>2</sub>). <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  1.56 (aliphatic chain, m, 2H), 2.23 (aliphatic chain and morpholine ring, m, 6H), 2.89 (aliphatic chain, m, 1H), 3.36 (aliphatic chain, m, 1H), 3.59 (morpholine ring and NH<sub>2</sub>, m, 6H), 4.74 (H-4, d,  $J$  = 4.3 Hz, 1H), 5.23 (H-3, d,  $J$  = 4.3 Hz, 1H), 6.50 (ArH, d,  $J$  = 8.4 Hz, 2H), 6.59 (ArH, d,  $J$  = 9.0 Hz, 2H), 6.97 (ArH, d,  $J$  = 8.4 Hz, 2H), 6.99 (ArH, d,  $J$  = 9.0 Hz, 2H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  24.4 (aliphatic chain), 38.5 (aliphatic chain), 53.6 (morpholine ring), 56.2 (aliphatic chain), 62.0 (C-4), 66.9 (morpholine ring), 81.9 (C-3), 114.7, 116.8, 121.9, 126.8, 129.1, 129.8, 147.0, 155.6 (aromatic carbons), 165.7 (CO,  $\beta$ -lactam). MS m/z = 416 [M<sup>+</sup>]. Anal. Calcd. for C<sub>22</sub>H<sub>26</sub>ClN<sub>3</sub>O<sub>3</sub>: C, 63.53; H, 6.30; N, 10.10%. Found: C, 63.67; H, 6.41; N, 10.06 %.

*4-(4-Aminophenyl)-3-(2,4-dichlorophenoxy)-1-(3-morpholino propyl)azetidín-2-one (6c)*

White solid (yield 67 %). mp 132-134 °C. IR (KBr, cm<sup>-1</sup>) 1743 (CO,  $\beta$ -lactam), 3332, 3409 (NH<sub>2</sub>). <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  1.63 (aliphatic chain, m, 2H), 2.29 (aliphatic chain and morpholine ring, m, 6H), 2.94 (aliphatic chain, m, 1H), 3.34 (aliphatic chain, m, 1H), 3.62 (morpholine ring and NH<sub>2</sub>, m, 6H), 4.79 (H-4, d,  $J$  = 4.4 Hz, 1H), 5.28 (H-3, d,  $J$  = 4.4 Hz, 1H), 6.52 (ArH, d,  $J$  = 8.2 Hz, 2H), 6.74 (ArH, d,  $J$  = 8.8 Hz, 1H), 6.95 (ArH, d,  $J$  = 8.8 Hz, 1H), 7.03 (ArH, d,  $J$  = 8.2 Hz, 2H), 7.13 (ArH, s, 1H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  24.1 (aliphatic chain), 38.4 (aliphatic chain), 53.4 (morpholine ring), 56.0 (aliphatic chain), 61.6 (C-4), 66.5 (morpholine ring), 82.1 (C-3), 114.7, 115.9, 121.5, 123.9, 126.9, 127.2, 129.4, 129.9, 147.2, 151.4 (aromatic carbons), 165.3 (CO,  $\beta$ -lactam). MS m/z = 450 [M<sup>+</sup>]. Anal. Calcd. for C<sub>22</sub>H<sub>25</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>3</sub>: C, 58.67; H, 5.60; N, 9.33%. Found: C, 58.72; H, 5.73; N, 9.37%.

*4-(4-Aminophenyl)-1-(3-morpholinopropyl)-3-(naphthalene-2-yloxy)azetidín-2-one (6d)*

White solid (yield 79 %). mp 152-154 °C. IR (KBr, cm<sup>-1</sup>) 1737 (CO,  $\beta$ -lactam), 3338, 3430 (NH<sub>2</sub>). <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  1.71 (aliphatic chain, m, 2H), 2.42 (aliphatic chain and morpholine ring, m, 6H), 2.99 (aliphatic chain, m, 1H), 3.46 (aliphatic chain, morpholine ring and NH<sub>2</sub>, m, 7H), 4.93 (H-4, d,  $J$  = 4.0 Hz, 1H), 5.50 (H-3, d,  $J$  = 4.0 Hz, 1H), 6.53 (ArH, d,  $J$  = 8.1 Hz, 2H), 6.95 (ArH, d,  $J$  = 8.6 Hz, 1H), 7.03 (ArH, s, 1H), 7.12 (ArH, d,  $J$  = 8.1 Hz, 2H), 7.26-7.69 (ArH, m, 5H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  24.4 (aliphatic chain), 38.5 (aliphatic chain), 53.6 (morpholine ring),

56.2 (aliphatic chain), 62.2 (C-4), 66.8 (morpholine ring), 81.8 (C-3), 108.9, 114.8, 118.5, 122.1, 124.0, 126.3, 126.8, 127.6, 129.3, 129.4, 129.8, 133.9, 146.9, 154.9 (aromatic carbons), 166.0 (CO,  $\beta$ -lactam). MS  $m/z = 431 [M^+]$ . Anal. Calcd. for  $C_{26}H_{29}N_3O_3$ : C, 72.37; H, 6.77; N, 9.74%. Found: C, 72.45; H, 6.89; N, 9.69 %.

*4-(3-Aminophenyl)-1-(3-morpholinopropyl)-3-phenoxyazetidin-2-one (6e)*

Off-white solid (yield 68 %). mp 117-119 °C. IR (KBr,  $cm^{-1}$ ) 1751 (CO,  $\beta$ -lactam), 3356, 3456 (NH<sub>2</sub>). <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  1.62 (aliphatic chain, m, 2H), 2.25 (aliphatic chain and morpholine ring, m, 6H), 2.95 (aliphatic chain, m, 1H), 3.47 (aliphatic chain, m, 1H), 3.63 (morpholine ring and NH<sub>2</sub>, m, 6H), 4.79 (H-4, d,  $J = 4.4$  Hz, 1H), 5.35 (H-3, d,  $J = 4.4$  Hz, 1H), 6.56 (ArH, d,  $J = 7.7$  Hz, 1H), 6.60 (ArH, d,  $J = 8.3$  Hz, 1H), 6.66 (ArH, s, 1H), 6.72 (ArH, d,  $J = 8.5$  Hz, 2H), 6.99 (ArH, t,  $J = 7.7$  Hz, 1H), 7.07 (ArH, t,  $J = 8.5$  Hz, 1H), 7.11 (ArH, t,  $J = 7.5$  Hz, 2H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  24.4 (aliphatic chain), 38.6 (aliphatic chain), 53.6 (morpholine ring), 56.2 (aliphatic chain), 62.3 (C-4), 66.9 (morpholine ring), 81.9 (C-3), 114.7, 115.4, 115.7, 118.9, 121.9, 129.1, 129.2, 134.2, 146.5, 157.1 (aromatic carbons), 166.1 (CO,  $\beta$ -lactam). MS  $m/z = 382 [M^+]$ . Anal. Calcd. for  $C_{22}H_{27}N_3O_3$ : C, 69.27; H, 7.13; N, 11.02%. Found: C, 69.45; H, 7.25; N, 10.95 %.

*4-(3-Aminophenyl)-1-(3-morpholinopropyl)-3-(naphthalene-2-yloxy)azetidin-2-one (6f)*

White solid purified by column chromatography (eluent 5:1 EtOAc/EtOH) (yield 59 %). mp 133-135 °C. IR (KBr,  $cm^{-1}$ ) 1751 (CO,  $\beta$ -lactam), 3348, 3440 (NH<sub>2</sub>). <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  1.67 (aliphatic chain, m, 2H), 2.34 (aliphatic chain and morpholine ring, m, 6H), 3.02 (aliphatic chain, m, 1H), 3.50 (aliphatic chain, morpholine ring and NH<sub>2</sub>, m, 7H), 4.90 (H-4, d,  $J = 4.3$  Hz, 1H), 5.52 (H-3, d,  $J = 4.3$  Hz, 1H), 6.50 (ArH, d,  $J = 7.9$  Hz, 1H), 6.65-7.69 (ArH, m, 10H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  24.3 (aliphatic chain), 38.7 (aliphatic chain), 53.5 (morpholine ring), 56.1 (aliphatic chain), 62.3 (C-4), 66.7 (morpholine ring), 81.9 (C-3), 109.3, 114.7, 115.5, 118.6, 118.9, 124.1, 126.4, 126.9, 127.6, 129.2, 129.3, 129.5, 133.9, 134.2, 146.5, 154.9 (aromatic carbons), 166.0 (CO,  $\beta$ -lactam). MS  $m/z = 431 [M^+]$ . Anal. Calcd. for  $C_{26}H_{29}N_3O_3$ : C, 72.37; H, 6.77; N, 9.74%. Found: C, 72.55; H, 6.85; N, 9.69 %.

## ***In vitro* anti-inflammatory activity [42]**

### **Principle of the assay**

The *in vitro* anti-inflammatory assay is based on the ability of macrophages to generate a strong inflammatory response when stimulated with antigens. Mouse immortalized macrophages (RAW 264.7 cell line) are stimulated by *E. coli* LPS and exposed to the test material for 24 hours. At the end of the incubation period, NO production is evaluated indirectly by measuring the accumulation of nitrite/nitrate, the stable end-products of NO oxidation, in the culture medium using a spectrophotometric method based on the Griess reaction. [42]

### **Cell line**

Mouse macrophages (RAW 264.7, Sigma-Aldrich, N° P6110401, Lot. 09I006), low passage number (<50).

### **Culture medium**

Complete medium: DMEM with L-glutamine (Dulbecco's Minimum Essential Medium, Pan Biotech Lot 974251) supplemented with penicillin-streptomycin (10,000 U/mL penicillin, 10 mg/mL streptomycin, Pan Biotech, Lot 20145123), and 10% inactivated calf serum (Pan Biotech, Lot P440008), pH 7.2, freshly prepared, stored no longer than 3 weeks.

### **Dilutions of the test material**

The test materials were diluted into dimethylsulfoxide (DMSO, Sigma-Aldrich) at the final concentration of 10 mM.

### **Controls**

Negative control: DMSO

Positive control: Dexamethasone at 1, 5, 10, 50, and 100  $\mu$ M in DMSO

### **Test procedure**

Cells were seeded into 48-well tissue culture plates at the concentration of  $1 \times 10^5$  cells/mL (200  $\mu$ L/well) for 24 hours at 37°C (5% CO<sub>2</sub>). At the end of the incubation period the culture medium was replaced by 200  $\mu$ L of medium containing the appropriate concentrations of the test materials, and cells were incubated at 37°C (5% CO<sub>2</sub>) for one hour. At the end of the incubation period, pro-inflammatory LPS from *E. coli* was added to the cell cultures (1  $\mu$ g/mL). The cells were then incubated at 37°C (5% CO<sub>2</sub>) for 24 hours.

### **Assessment of NO release**

NO release was measured in the culture supernatant by the Griess reaction. 100  $\mu$ l of the supernatants were transferred into the wells of a 96-well tissue culture plate, and 100  $\mu$ l of the Griess modified reagent (Sigma-Aldrich) were added in each well. After a 15 min period at room temperature, the Optical Density (OD) of each well was read at 540 nm by a fluorescence-luminescence reader Infinite M200 Pro (Tecan). The results obtained for wells treated with the test material were compared to those of untreated control wells (DMSO, 100% viability) and converted to percentage values.

### **Assessment of cell viability**

In parallel to the assessment of NO release, cell viability was measured to validate the assay. The WST-1 vital dye reagent was used to measure cell mitochondrial respiration. For this purpose, the culture medium was decanted and 100 $\mu$ l of WST-1 reagent (1/10 dilution) were added in each well. After a 30-min incubation period at 37°C (5% CO<sub>2</sub>), the Optical Density (OD) of each well was read at 450 nm by a fluorescence-luminescence reader Infinite M200 Pro (TECAN). The results obtained for wells treated with the test material were compared to those of untreated control wells (DMSO, 100% viability) and converted to percentage values.

### **Calculation of the IC<sub>50</sub>**

Inhibition of NO release and inhibition of cell viability were expressed as percentages as compared to the negative controls:

$$\text{Percentage of NO release} = \frac{100 \times (\text{OD of test well} - \text{OD of blank})}{\text{OD of DMSO control} - \text{OD of blank}}$$

$$\text{Percentage of Cell viability} = \frac{100 \times (\text{OD of test well} - \text{OD of blank})}{\text{OD of DMSO control} - \text{OD of blank}}$$

The concentrations of the test material causing respectively a 50% decrease of NO release (IC<sub>50-NO release</sub>) and a 50% decrease of cell viability (IC<sub>50-cell viability</sub>) were calculated using software Tablecurve Version 2.0. The anti-inflammatory ratio corresponded to the ratio between the anti-inflammatory activity and the toxicity, and expressed as follows:

Anti-inflammatory ratio =  $IC_{50\text{-cell viability}} / IC_{50\text{-NO release}}$

## Molecular docking studies

Computer-simulated docking studies were accomplished by the AutoDock 4.2 software. [43] Lamarckian Genetic Algorithm of the AutoDock 4.2 program was used as the search algorithm. The Graphical User Interface program AutoDock Tools 1.5.6 (ADT) were used to prepare, run, and analyze the docking simulations. Molecular docking of compounds was performed with the published crystal structure (PDB ID: 4NOS (human inducible nitric oxide synthase)) by the Auto-Dock Tool 1.5.6. All two dimensional (2D) structures of the compounds were built using the ChemDraw program (ChemDraw Ultra 10.0, Cambridge soft.), and then moved into the Hyperchem 8.0 software (HyperChem, Release 8.0 for Windows, Molecular Modeling System: HyperCube, 2007). Molecules were subjected to energy minimization with MM<sup>+</sup> force field and then PM3 semi-empirical technique. Then the partial charges of atoms were calculated by the GasteigerMarsili procedure implemented in the AutoDock Tools package. The non-polar hydrogens of compounds were merged. The crystal structure of protein was taken from Protein Data bank ([www.rcsb.org](http://www.rcsb.org)). All bound water and ligands were eliminated from the protein, and polar hydrogen atom were added to the protein as it was required for the electrostatics interactions, and then non-polar hydrogen atoms were merged together. In all dockings, a grid map with 60 grid points in the X, Y, and Z directions was built. Among the three different search algorithms offered by AutoDock 4.2, the Lamarckian genetic algorithm (LGA) approach was applied. For all docking procedures, 150 independent runs with the step sizes of 0.2 Å for translations and 5° for orientations and torsions were considered. For the Lamarckian GA method, a maximum number of  $25 \times 10^5$  energy evaluations; 27,000 maximum generations; a gene mutation rate of 0.02; and a cross-over rate of 0.8 were used. At the end of docking, the structures were ranked by energy. Ligand-receptor interactions were all visualized on the basis of docking results using Discovery Studio Visualizer 4.0 and Ligplus2012.

## Conclusions

In this study, some new monocyclic  $\beta$ -lactam derivatives bearing a morpholine ring substituent on the nitrogen have been synthesized and evaluated for anti-inflammatory activities.

Some of the morpholino- $\beta$ -lactam hybrids showed higher anti-inflammatory responses than the corticosteroid dexamethasone. The anti-inflammatory activity was observed to be dependent on the substituents at the C3 and C4 centers of the lactam ring. Members of the **3a-o** and **5a-h** series exhibit higher  $\Delta G_{\text{binding}}$  than the **6a-e** series. The higher lipophilicity and electron-withdrawing characters had positive effect for anti-inflammatory activity and score docking. These results suggest the possibility of carrying out further studies on the mechanism of action of our compounds.

### Conflict of interest

The authors declare that there is no conflict of interests.

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