

Synthesis of anticancer β -lactams: mechanism of action

Bimal K. Banik,* Frederick F. Becker and Indrani Banik

The University of Texas M.D. Anderson Cancer Center, Department of Molecular Pathology, Unit 89, 1515 Holcombe Boulevard, Houston, TX 77030, USA

Received 22 November 2003; revised 8 March 2004; accepted 16 March 2004

Available online 10 April 2004

Abstract—Synthesis of the *trans* 1-*N*-chrysenyl and 1-*N*-phenanthrenyl 3-acetoxy-4-phenyl-2-azetidinones has been achieved. Microwave-assisted reaction has proved useful in the synthesis of these compounds. Cell growth inhibition study has indicated selective anticancer activity against two leukemia and colon carcinoma cell lines. A mechanistic correlation of their anticancer activity has been described. Striking G₂ blockade that is clearly distinct in cell cycle analysis and demonstrated only in sensitive cell lines has been observed. They do not induce apoptosis in sensitive or resistant lines. They also do not inhibit topoisomerases. Ames test has shown they are nonmutagenic.
© 2004 Published by Elsevier Ltd.

1. Introduction

In previous publications, we demonstrated synthesis and biological evaluation of some derivatives of polyaromatic amines, which were open-chain amides to which the polycyclic residue was bound (e.g., **1** and **2**, Fig. 1).¹ From the literature, we are aware that conformationally constrained molecules often have a greater effect on biological properties when compared to the relatively flexible open-chain compounds.² Therefore, we anticipated that conformationally constrained analogues of

our open-chain diamides (**1** and **2**) may increase potency. Structures **1** and **2** suggest that a ring formation using N₁ and C₄ would result in β -lactam **3**. Our research in this field has culminated in the synthesis of a few β -lactams derived from polyaromatic imines.³ A preliminary structure–activity study has revealed that *trans* 1-*N*-chrysenyl and 1-*N*-phenanthrenyl 3-acetoxy-4-aryl-2-azetidinones have potent, selective anticancer activity. Because β -lactams are widely and safely used as antibacterials without significant toxicity, studies of these molecules as new and novel anticancer agents would be timely. In this paper, we describe mechanistic correlation of their anticancer activity for the first time and demonstrate a remarkable G₂ blockade that is clearly distinct in cell cycle analysis. In addition, synthesis of these agents using microwave irradiation is also described here.

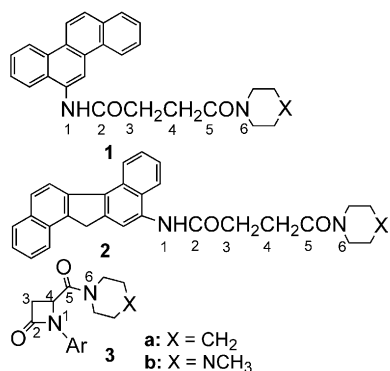


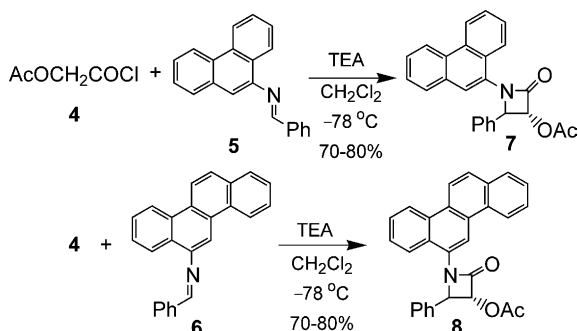
Figure 1.

Keywords: β -Lactams; Anticancer agents; Cell cycle; Apoptosis.

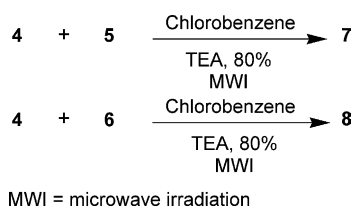
* Corresponding author. Tel.: +1-713-792-7749; fax: +1-713-792-5940;
e-mail: bbanik@mail.mdanderson.org

1.1. Synthesis of β -lactams

The two β -lactams **7** and **8** were prepared by following Staudinger reaction. Cycloaddition of imines **5** and **6** with acetoxy acetyl chloride **4** in the presence of triethylamine afforded *trans*- β -lactams **7** and **8**, respectively (Scheme 1). Although formation of β -lactams using the Staudinger reaction was discovered more than 90 years ago, surprisingly, there has not been a precedent recorded in the literature regarding the use of tetracyclic or pentacyclic aromatic systems in imine components. Interestingly, the formation of *trans*- β -lactams as seen in



Scheme 1.



Scheme 2.

the present investigation also has not been described in the literature. Some previous studies were directed at the formation of *trans*- β -lactams. However, the experimental conditions in those investigations were clearly different from those in the present one. For example, synthesis of some *trans*- β -lactams was achieved using high-power microwave irradiation and changing the order in which the reagents were added.^{4c,d} Furthermore, in two cases, *trans*- β -lactams were formed in low yield as the only isolated products using cyclic imines, but they were not derived from an aromatic amine.^{4a,b}

The use of domestic microwave in organic synthesis is well known.⁵ In conformity with earlier hypothesis, irradiation of a solution of imines **5** and **6** in chlorobenzene in the presence of acetoxy acetyl chloride **4** and triethylamine using a domestic microwave oven afforded *trans*- β -lactams **7** and **8**, respectively, in comparable yield (Scheme 2).⁶ *trans*- β -Lactams **7** and **8** were also obtained when the above reaction mixtures were heated in an oil-bath (120 °C) for 30 min using a stirrer and reflux condenser.

An unconventional experimental setup for conducting this reaction to take special advantages of the specific nature of microwave energy was used. A large size Erlenmeyer flask was the reaction vessels in unmodified

domestic microwave oven.^{5c,d} Polar solvents like chlorobenzene and DMF can be used with success. However, chlorobenzene proved to be the preferred solvent in this case. The boiling point of these solvents is much higher than the projected reaction temperature. β -Lactams **7** and **8** was obtained after 4–5 min of irradiation in the microwave when chlorobenzene was the solvent. The temperature of the reaction vessel was maintained at 100–110 °C by a proper adjustment of the ‘on–off’ cycle and a ‘heat sink’.^{5c,d} Microwave energy is absorbed by all of the polar molecules at the same time effectively, therefore no stirrers was needed. Reflux condenser was not required since liquids were maintained below their boiling points under this condition. Therefore, an easy access of these β -lactams was realized using microwave-induced method.

The mechanism of β -lactam formation has been investigated extensively. However, the rationale for the observed diastereoselectivity and enantioselectivity remains unknown. It has been shown that the stereoselectivity depends on a number of factors: the structure of the imine, acid-chloride (equivalent) sequence of reagent addition, solvent, temperature, and bases. It is conceivable that the mechanism of β -lactam formation reaction by Staudinger reaction is not only very complex, but also unique since it varies considerably with minor structural changes and conditions. Certainly, any of the postulated mechanisms may be operative, and there may be a continuum of mechanisms acting in concert to promote the reaction depending on the particular method employed.⁷

1.2. In vitro cytotoxicity of the β -lactams

These β -lactams **7** and **8** were tested using nine human cancer cell lines with cisplatin and diamide compounds **1b** and **2b** as controls; results are given in Table 1.

2. Mechanism of action

Because of the presence of the cyclic four-membered amide connected to the polycyclic ring systems, an effort to define their mechanism of action would be highly important. Despite a number of synthetic efforts, a relatively small amount of research has been focused on the use of compounds related to PAHs as anticancer agents. For example, Bair et al.⁸ reported a close correlation between antitumor activity and the shape of the polycyclic aromatic system. However, his group did not make

Table 1. In vitro cytotoxicity of β -lactam compounds on human cancer cell lines (μ M)

Compounds	BRO	MCF-7	MDA-231	OVCAR-3	SKOV-3	PC-3	HL-60	K-562	HT-29
Cisplatin	7.66	10.05	12.33	3.99	5.99	4.66	1.66	2.33	16.99
1b	33.64	40.0	12.23	18.11	11.05	27.29	9.41	12.70	16.70
2b	3.9	4.3	5.1	4.1	3.9	3.5	2.1	1.9	3.9
7	10.48	10.09	12.49	18.0	18.00	9.3	5.21	4.0	10.49
8	10.84	9.81	11.98	4.17	6.88	16.32	3.64	4.33	5.66

All of the in vitro cytotoxicity assays were performed in the Pharmacology and Analytic Core Laboratory of our Cancer Center as described previously.¹ In summary, an MTT assay was carried out using the nine human cancer cell lines.

a definitive correlation between the ability of these compounds to bind to DNA and their cytotoxic activity. Bair's group developed benzylic aminopropanediols from a structure–activity relationship study. These amino propanediols are believed to interact with DNA via intercalation and to be topoisomerase II inhibitors. The antitumor activity of two of the most active naphthalimides amonafide and mitonafide, has been shown to have to be due to intercalation.⁹ To improve its potency, naphthalene was modified to anthracene to serve as the chromophore. The resulting analogue, azonafide showed increased cytotoxic potency over naphthalene-based compounds.¹⁰ Not all azonafide derivatives, which have characteristic mechanistic properties when compared with existing classes of DNA intercalators, localize in the nucleus. Other differences with existing DNA intercalators, such as mitoxantrone,¹¹ include a lack of inhibition of topoisomerase II enzymes at equicytotoxic concentrations. Another approach to improving cellular cytotoxic potency has been adopted by linking two naphthalimide or anthralimide groups with a polyamine bridge. Other studies concerning the target for several newly synthesized agents have been published by Denny and co-workers¹² The synthesis and mechanism of action of β -lactams as different types of biologically active compounds have been investigated.^{13,14} However, studies directed to define the mechanism of action of β -lactams as anticancer agents has not been explored. The active compounds **7** and **8** may follow one of these mechanisms as described above or it may act on cancer cells through a novel mechanism. To define the mechanism of action of our two potent compounds, the studies described below were performed.

2.1. Mutagenicity assays

Mutagenicity assays were performed by BioReliance Corporation (Rockville, MD) as study AA65FL-FM.501.BTA. Compounds **7** and **8** were examined using the Salmonella Plate Incorporation, Mutagenicity Assay. The tester strains used were Salmonella typimurium histidine auxotrophs TA98 and TA100 as described by Ames et al.¹⁵ Tester strain TA98 was reverted from auxotrophy to prototrophy by frame shift mutagens. Tester strain TA100 is reverted by mutagens that cause both frame shift and base pair substitution mutations.

Compounds **7** and **8** were exposed via the plate incorporation methodology originally described by Ames et al. and updated by Maron and Ames.¹⁶ Each one of the compound was tested at 10 dose levels along with appropriate vehicle control and positive controls with tester strains TA98 and TA100 in the presence and absence of Aroclor-induced rat liver S9. All dose levels of test compounds, vehicle control and positive controls, were plated in duplicate.

For each test compound to be evaluated positive, it must have caused a dose-related increase in the mean revertants per plate for at least one tester strain over a minimum of two increasing concentrations of the test

article. Data sets for tester strains TA98 and TA100 were judged to be positive if the increase in mean revertants at the peak of the dose response was equal to or greater than twice the mean vehicle control value.

The results of the mutagenicity assay indicated that under the conditions of this study, neither **7** nor **8** demonstrated a positive response with these tester strains at any concentration in either the presence or absence of Aroclor-induced rat liver S9, indicating that neither of these compounds demonstrate any mutagenicity. These findings support the potential use of these compounds as antitumor agents and eliminate potential concerns arising from the presence of the phenanthrene and chrysene nuclei.

2.2. Apoptosis

To determine whether exposure to **7** and/or **8** induced DNA cleavage in sensitive and/or resistant tumor cell lines, the APO-BRDU assay was carried out. As shown in Tables 2 and 3, **8** demonstrated no significant increase in BRDU insertion into the DNA of HL-60 cells exposed for 24 h. In two similar runs **7** also failed to demonstrate an increase in BRDU insertion. In all of these experiments, concentrations approximately twice that required for the IC₅₀ of **7** and three times greater than that for **8** were used. In one of these experiments, MDA-231, a human breast cancer line that was resistant in vitro to the effects of **8**, was tested for BRDU insertion. As expected, a significant increase was not detected. These results confirmed scanning electron microscopy studies of exposed cells (data not presented) that confirmed the lack of alterations in these cell lines, which are associated with the induction of apoptosis in the target cells.

2.3. Interactions with topoisomerases

For the purposes of these analytic procedures, we used the assay kits provided by TOPOGEN, Inc.

Table 2. BRDU insertion in sensitive tumor cells^a

Negative cells (kit)	(2)	1.1%	
Positive cells (kit)	(2)	38.5%	
Cell lines	0 h	6 h	24 h
HL-60 (untreated) (2)	2.9%	2.9%	2.3%
HL-60 (8 , 10 μ g/mL) (2)	2.7%	2.4%	4.2%
HL-60 (7 , 10 μ g/mL) (2)	3.2%	2.9%	3.5%

^a Percentage of cells demonstrating BRDU insertion.

Table 3. BRDU insertion in sensitive/resistant tumor cells

Negative cells (kit)	(1)	0.9%	
Positive cells (kit)	(1)	46%	
Cell lines	0 h	6 h	24 h
HL-60 (8 , 10 μ g/mL) (1)	6.6%	6.6%	4.7%
MDA-231 (no treatment) (1)	6.7%	6.6%	4.7%
MDA-231 (7 , 10 μ g/mL) (1)	5.4%	8.0%	10.0%

(Columbus, Ohio). These kits contained all of the reagents required for routine detection of topoisomerase I and II with DNA markers for detection of enzymatic action and standard topoisomerase inhibitors. The activity of **7** and **8** and was determined using these systems.

Using approximately three times the concentration of **8** and two times the concentration of **7** produced IC₅₀ in the HL-60 line; no inhibition of either topoisomerase I or topoisomerase II was detected. Therefore, there was no evidence that the active β -lactams **8** and **7** had their cytotoxic activity in sensitive cell lines through interaction with DNA or DNA-related enzyme systems. These compounds failed to increase BRDU insertion into DNA and display any inhibitory action toward the two topoisomerase systems examined. In a broad sense therefore, the failure to induce apoptosis was also consistent with these findings because in many instances, this phenomenon was the putative result of such DNA interactions.

2.4. Cell cycle activity

In parallel with the apoptosis studies described above, we utilized insertion of propidium iodine (the marker for DNA content) as a marker of the cell cycle. This cytometric determination was calibrated separately and calculated for a related program to determine the percentage of cells in each phase of the cell cycle. In each instance the treated cells were compared with that of control HL-60 cells for the percentage of cells in each cell cycle compartment over a period of 24 h. HL-60 cells treated with 10 μ g of **8** demonstrated a consistent increase in the percentage of cells in the G₂ phase that was particularly striking at 24 h. While the average percentage of untreated cells in G₂ was 17.2%, that of cells treated with **8** and **7** was 38.4% and 23.6%, respectively (Table 4). To determine whether alteration of the cell cycle occurred in a cell line that had repeatedly demonstrated resistance to the in vitro effect of **8**, we examined the human breast cancer cell line MDA-231 (Table 5). While HL-60 cells treated with **8** (10 μ g) again demonstrated a striking increase in the G₂ phase at 24 h (34.1%), no increase was detected in MDA-231, which was in effect identical to that in untreated MDA-231 (approximately 14%).

Table 4. Analysis of cell cycle studies^a

Cell lines	Time of exposure (h)	G ₁	S	G ₂
HL-60 (no treatment)	0	43.3%	42.8%	13.9%
	6	41.6%	44.2%	16.2%
	24	45.0%	37.9%	17.2%
HL-60 (8 , 10 μ g/mL)	0	44.8%	44.6%	13.2%
	6	27.1%	57.8%	16.9%
	24	8.9%	52.4%	38.4%
HL-60 (7 , 10 μ g/mL)	0	45.3%	43.2%	11.5%
	6	31.4%	51.3%	17.4%
	24	18.1%	58.4%	23.6%

^a The results represent the average of two separate runs.

Table 5. Analysis of cell cycle studies

Cell lines	Time of exposure (h)	G ₁	S	G ₂
HL-60 (8 , 10 μ g/mL)	0	38.0%	48.7%	13.3%
	6	26.8%	50.4%	22.5%
	24	11.0%	54.9%	34.1%
MDA-231 (no compound)	0	58.2%	28.1%	13.7%
	6	58.0%	29.2%	12.9%
	24	59.4%	26.2%	14.4%
MDA-231 (7 , 10 μ g/mL)	0	60.9%	26.7%	12.4%
	6	49.9%	32.2%	17.1%
	24	48.5%	37.6%	13.9%

3. Conclusion

Although the mechanism of action of the lead compounds has not been totally established, our research on cell cycle analysis offers intriguing possibilities. In the absence of effect upon DNA or DNA enzyme systems they produced a striking G₂ blockade. This 'check point' in the cell cycle has attracted a considerable amount of attention recently.¹⁷ Studies are now underway to determine the exact site of this action and to utilize it in an effort to modify our β -lactams to obtain more potent compounds.

4. Experimental

4.1. General methods

All of the solvents and reagents were obtained from commercial sources and used without purification. Reactions were monitored by TLC using pre-coated silica gel aluminum plates containing a fluorescence indicator. Chemical shifts of ¹H NMR spectra were given in parts per million with respect to TMS, and the coupling constant *J* was measured in Hz. The data are reported as follows: chemical shifts, multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet). ¹H NMR spectra were recorded in CDCl₃ using tetramethylsilane as an internal standard. IR spectra were expressed as wave numbers (cm⁻¹).

4.2. Preparation of imine (general procedure)

To a solution of the amine (10 mmol) in toluene (40 mL) was added benzaldehyde (12 mmol) and the mixture was refluxed overnight using a Dean–Stark water separator (monitored by TLC). When the reaction was over, toluene was evaporated under reduced pressure, and the crude product was used as such for the next reaction.

4.3. Preparation of β -lactam (general procedure)

A solution consisting of acetoxy acetyl chloride (1.5 mmol) in dichloromethane (10 mL) was added drop wise to a stirred solution containing imine (1 mmol) and distilled triethylamine (3 mmol) in dry dichloromethane (10 mL) at –78 °C. The reaction mixture was then stirred

overnight at room temperature, washed with saturated sodium bicarbonate solution (10 mL), dilute hydrochloric acid (10%, 10 mL), brine (10 mL), dried with anhydrous sodium sulfate, and evaporated to obtain the crude product. Proton NMR was performed to calculate the ratio of the isomeric β -lactams. The pure product (70–80%) was then isolated via column chromatography over silica gel using ethyl acetate–hexanes (1:4) as the solvent.

4.4. Microwave-assisted preparation of the β -lactam (general procedure)

The same amount of imine, acid chloride, triethylamine along with chlorobenzene (5 mL) was placed in an Erlenmeyer flask (125 mL capacity). The flask was then capped with a glass funnel and placed in a microwave oven (G. E. Model, 1450 W). A 500 mL beaker containing 200 mL of water was placed in the oven next to the reaction flask to serve as a heat sink. The mixture was irradiated for 3 min at intervals of 1 min each. After the usual work up as described above, the β -lactam was isolated (80% yield).

4.5. *trans*-N-(9-Phenanthrenyl)-3-acetoxy-4-phenyl-2-azetidine-2-one (7)

Mp 152–154 °C; IR cm^{-1} (CH_2Cl_2) 1754, 1625, 1599, 1528, 1498, 1455, 1401, 1369, 1280, 1219; ^1H NMR (CDCl_3) δ (ppm) 2.27 (s, 3H), 5.43–5.44 (d, $J = 1.92$ Hz, 1H), 5.70–5.71 (d, $J = 1.96$ Hz, 1H), 7.27–7.29 (m, 3H), 7.40–7.42 (m, 2H), 7.52–7.63 (m, 3H), 7.73–7.78 (m, 3H), 8.29–8.32 (m, 1H), 8.62–8.64 (d, 1H), 8.70–8.73 (m, 1H); Anal. Calcd for $\text{C}_{25}\text{H}_{19}\text{NO}_3$: C, 78.72; H, 5.02; N, 3.67. Found: C, 78.69; H, 5.17; N, 3.70.

4.6. *trans*-N-(6-Chrysenyl)-3-acetoxy-4-phenyl-2-azetidine-2-one (8)

Mp 174–176 °C; IR cm^{-1} (CH_2Cl_2) 1755, 1595, 1515, 1486, 1456, 1440, 1394, 1373, 1314, 1283, 1219; ^1H NMR (CDCl_3) δ (ppm) 2.36 (s, 3H), 5.54–5.55 (d, $J = 1.91$ Hz, 1H), 5.77–5.78 (d, $J = 1.94$ Hz, 1H), 7.26–7.31 (m, 3H), 7.46–7.49 (m, 2H), 7.60–7.71 (m, 2H), 7.77–7.80 (m, 2H), 7.95–7.99 (m, 2H), 8.38–8.41 (m, 1H), 8.45 (s, 1H), 8.50–8.53 (d, 1H), 8.63–8.66 (d, 1H), 8.79–8.82 (m, 1H); Anal. Calcd for $\text{C}_{29}\text{H}_{21}\text{NO}_3$: C, 80.72; H, 4.91; N, 3.25. Found: C, 80.69; H, 4.89; N, 3.19.

Acknowledgements

We gratefully acknowledge the partial financial support for this research project from The University of Texas M. D. Anderson Cancer Center. We are also thankful for NIH Cancer Center Support Grant 5-P30-CA16672-25, in particular, the shared resources of the Pharmacology and Analytical Center Facility.

References and notes

- (a) Becker, F. F.; Banik, B. K. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2877–2880; (b) Becker, F. F.; Mukhopadhyay, C.; Hackfeld, L.; Banik, I.; Banik, B. K. *Bioorg. Med. Chem.* **2000**, *8*, 2693–2699; (c) Banik, B. K.; Becker, F. F. *Bioorg. Med. Chem.* **2001**, *9*, 593–605; (d) Banik, B. K.; Becker, F. F. *Curr. Med. Chem.* **2001**, *8*, 1513–1533; (e) Banik, B. K., Ed.; *Curr. Med. Chem.* **2001**, *8*, 1343–1553; (a) Also see: Becker, F. F.; Banik, B. K. U.S. Patent 6,015,811, 2000; (b) Becker, F. F.; Banik, B. K. U.S. Patent 6,184,224, 2001; (c) Becker, F. F.; Banik, B. K. U.S. Patent 6,362,200, 2002.
- (a) Lin, T. H.; Rogers, T. S.; Hill, D. L.; Simpson-Herren, L.; Farnell, D. R.; Kochhar, D. M.; Alam, M.; Brouillette, W. J.; Muccio, D. D. *Toxicol. Appl. Pharmacol.* **1996**, *139*, 310–316; (b) Garratt, P. J.; Vonhoff, S.; Rowe, S. J.; Sugden, D. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1559–1564; (c) Mathe-Allainmat, M.; Gaudy, F.; Sicic, S.; Dangy-Caye, A.-L.; Shen, S.; Bremont, B.; Benatalah, Z.; Langlois, M.; Renard, P.; Delagrangue, P. *J. Med. Chem.* **1996**, *39*, 3089–3095; (d) Anderson, A.; Boyd, A. C.; Clark, J. K.; Fielding, L.; Gemmell, D. K.; Hamilton, N. M.; Maidment, M. S.; May, V.; McGuire, R.; McPhail, P.; Sansbury, F. H.; Sundaram, H.; Taylor, R. *J. Med. Chem.* **2000**, *43*, 4118–4125; (e) Barboni, L.; Lambertucci, C.; Appendino, G.; Vander Velde, D. G.; Himes, R. H.; Bombardelli, E.; Wang, M.; Snyder, J. P. *J. Med. Chem.* **2001**, *44*, 1576–1587; (f) Hernandez, A. I.; Balzarini, J.; Karlsson, A.; Camarasa, M. J.; Perez, M. J. *J. Med. Chem.* **2002**, *45*, 4254–4263; (g) Selvakumar, N.; Srinivas, D.; Khera, M. K.; Kumar, M. S.; Mamidi, R. N. V. S.; Sarnaik, H.; Charavaryamath, C.; Rao, B. S.; Raheem, M. A.; Das, J.; Iqbal, J.; Rajagopalan, R. *J. Med. Chem.* **2002**, *45*, 3953–3962; (h) Ullrich, T.; Krich, S.; Binder, D.; Mereiter, K.; Anderson, D. J.; Meyer, M. D.; Pyerin, M. *J. Med. Chem.* **2002**, *45*, 4047–4054.
- Banik, I.; Becker, F. F.; Banik, B. K. *J. Med. Chem.* **2003**, *46*, 12–15; For some of our research on β -lactams, see: (a) Ghatak, A.; Becker, F. F.; Banik, B. K. *Heterocycles* **2000**, *53*, 2769–2772; (b) Banik, B. K.; Ghatak, A.; Becker, F. F. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2179–2181; (c) Banik, B. K.; Becker, F. F. *Tetrahedron Lett.* **2000**, *41*, 6551–6554; (d) Ng, S.; Banik, I.; Okawa, A.; Becker, F. F.; Banik, B. K. *J. Chem. Res.* **2001**, 118–119; (e) Dasgupta, S. K.; Banik, B. K. *Tetrahedron Lett.* **2002**, *43*, 9445–9447; (f) Banik, B. K.; Samajdar, S.; Banik, I. *Tetrahedron Lett.* **2003**, *44*, 1699–1701; (g) Banik, B. K.; Banik, I.; Hackfeld, L. *Heterocycles* **2003**, *59*, 505–508; (h) Banik, B. K.; Samajdar, S.; Banik, I.; Hann, J. *Heterocycles* **2003**, *61*, 97–100; (i) Banik, B. K.; Adler, D.; Nguyen, P.; Srivastava, N. *Heterocycles* **2003**, *61*, 101–104; (j) Banik, B. K.; Banik, I.; Samajdar, S.; Wilson, M. *Heterocycles* **2003**, *63*, 283–296; (k) Banik, B. K. Ed.; β -Lactams: Synthesis, Stereochemistry, Synthons and Biological Evaluation. *Curr. Med. Chem.*, in press.
- (a) Alcaide, B.; Vicente-Rodriguez, A. *Tetrahedron Lett.* **1999**, *40*, 2005–2006; (b) Afonso, A.; Rosenblum, S. B.; Puar, M. S.; McPhail, A. T. *Tetrahedron Lett.* **1998**, *39*, 7431–7434; (c) Endo, M.; Droghini, R. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2483–2486; (d) Bose, A. K.; Banik, B. K.; Manhas, M. S. *Tetrahedron Lett.* **1995**, *36*, 213–216.
- Microwave activation has become very popular and useful technology in organic and medicinal chemistry. For some recent examples, see: (a) Perreux, L.; Loupy, A. *Tetrahedron* **2001**, *57*, 9199–9223; (b) Lidstrom, P.; Tierney, J.; Wathey, B.; Westman, J. *Tetrahedron* **2001**, *57*, 9225–9287; (c) Bose, A. K.; Manhas, M. S.; Ganguly, S. N.; Sharma, A. H.; Banik, B. K. *Synthesis* **2002**,

- 1578–1591; (d) Bose, A. K.; Banik, B. K.; Lavlinskaia, N.; Jayaraman, M.; Manhas, M. S. *Chemtech* **1997**, 27, 18–24, and references cited therein.
6. One of us was extensively involved in the microwave-assisted β -lactam synthesis. For example, see: (a) Bose, A. K.; Manhas, M. S.; Ghosh, M.; Shah, M.; Raju, V. S.; Bari, S. S.; Newaz, S. N.; Banik, B. K.; Barakat, K. J.; Chaudhury, A. G. *J. Org. Chem.* **1991**, 5, 968–970; (b) Banik, B. K.; Manhas, M. S.; Kaluza, Z.; Barakat, K. J.; Bose, A. K. *Tetrahedron Lett.* **1992**, 33, 3603–3606; (c) Banik, B. K.; Manhas, M. S.; Newaz, S. N.; Bose, A. K. *Bioorg. Med. Chem. Lett.* **1993**, 3, 2363–2368; (d) Bose, A. K.; Banik, B. K.; Barakat, K. J.; Manhas, M. S. *Synlett* **1993**, 575–577; (e) Bose, A. K.; Manhas, M. S.; Banik, B. K.; Robb, E. W. *Res. Chem. Interim.* **1994**, 20, 1–20; (f) Banik, B. K.; Manhas, M. S.; Robb, E. W.; Bose, A. K. *Heterocycles* **1997**, 405; (g) Banik, B. K.; Jayaraman, M.; Srirajan, V.; Manhas, M. S.; Bose, A. K. *J. Ind. Chem. Soc.* **1997**, 74, 951; (h) Banik, B. K.; Barakat, K. J.; Wagle, D. R.; Manhas, M. S.; Bose, A. K. *J. Org. Chem.* **1999**, 64, 5746–5753.
7. (a) Arrieta, A.; Lecea, B.; Cossio, F. P. *J. Org. Chem.* **1998**, 63, 5869–5876; (b) Cossio, F. P.; Arrieta, A.; Lecea, B.; Ugalde, J. M. *J. Am. Chem. Soc.* **1994**, 116, 2085–2093; (c) Cossio, F. P.; Ugalde, J. M.; Lopez, X.; Lecea, B.; Palomo, C. *J. Am. Chem. Soc.* **1993**, 115, 995–1004; (d) Hegedus, L. S.; Montgomery, J.; Narukawa, Y.; Snustad, D. C. *J. Am. Chem. Soc.* **1991**, 113, 5784–5791; (e) Lopez, R.; Sordo, T. L.; Sordo, J. A.; Gonzalez, J. *J. Org. Chem.* **1993**, 58, 7036–7037; (f) Lynch, J. E.; Riseman, S. M.; Laswell, W. L.; Tschaen, D. M.; Volante, R. P.; Smith, G.; Shinkai, I. *J. Org. Chem.* **1989**, 54, 3792–3796; (g) Just, G.; Ugolini, A.; Zamboni, R. *Synth. Commun.* **1979**, 9, 117–121; (h) Doyle, T. W.; Belleau, B.; Luh, B. Y.; Ferrari, C. F.; Cunningham, M. P. *Can. J. Chem.* **1977**, 55, 468–507; (i) Bose, A. K.; Chiang, Y. H.; Manhas, M. S. *Tetrahedron Lett.* **1972**, 4091–4094.
8. Bair, K. W.; Andrews, C. W.; Tuttle, R. L.; Knick, V. C.; Cory, M.; McKee, D. *J. Med. Chem.* **1991**, 34, 1983–1990.
9. (a) Malviya, V. K.; Liu, P. Y.; Alberts, D. S.; Surwit, E. A.; Craig, J. B.; Hanningan, E. V. *Am. J. Clin. Oncol.* **1992**, 15, 41–44; (b) Rosell, R.; Carles, J.; Abad, A.; Ribelles, N.; Barnadas, A.; Benavides, A.; Martin, M. *Invest. New Drugs* **1992**, 10, 171–175.
10. (a) Sami, S. M.; Dorr, R. T.; Alberts, D. S.; Remers, W. A. *J. Med. Chem.* **1993**, 36, 765–770; (b) Sami, S. M.; Dorr, R. T.; Solymion, A. M.; Alberts, D. S.; Remers, W. A. *J. Med. Chem.* **1995**, 38, 983–993.
11. Fukushima, T.; Kawai, Y.; Nakayama, T.; Yamaguchi, T.; Yoshida, A.; Urasaki, Y.; Imamura, S.; Kamiya, K.; Tsutani, H.; Ueda, T.; Nakamura, T. *Oncol. Res.* **1996**, 8, 95–100.
12. (a) Palmer, B. D.; Rewcastle, G. W.; Atwell, G. J.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1988**, 31, 707–712; (b) Denny, W. A.; Rewcastle, G. W.; Ganguley, B. C. *J. Med. Chem.* **1990**, 33, 814–819; (c) Lee, H. H.; Palmer, B. D.; Boyd, M.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1992**, 35, 258–266; (d) Cherubim, P.; Deady, L. W.; Dorkos, M.; Qazi, N. H.; Baguley, B. C.; Denny, W. A. *Anti-Cancer Drug Design* **1993**, 8, 429–438.
13. For the synthesis of biologically active compounds using β -lactams: (a) Manhas, M. S.; Banik, B. K.; Mathur, A.; Vincent, J. E.; Bose, A. K. *Tetrahedron, Symposium in Print* **2000**, 56, 5587–5601; (b) Bose, A. K.; Banik, B. K.; Mathur, C.; Wagle, D. R.; Manhas, M. S. *Tetrahedron, Symposium in Print* **2000**, 56, 5603–5619; (c) Ojima, I. *Acc. Chem. Res.* **1995**, 28, 383–389; (d) Banik, B. K.; Manhas, M. S.; Bose, A. K. *J. Org. Chem.* **1994**, 59, 4714–4716; (e) Banik, B. K.; Manhas, M. S.; Bose, A. K. *J. Org. Chem.* **1993**, 58, 307–309; (f) Southgate, R.; Branch, C.; Coulton, S.; Hunt, E. In *Recent Progress in the Chemical Synthesis of Antibiotics and Related Microbial Products*; Lukacs, G., Ed.; Springer: Berlin, 1993; Vol. 2; (g) Kidwai, M.; Sapra, P.; Bhushan, K. R. *Curr. Med. Chem.* **1999**, 6, 195–215; (h) Bose, A. K.; Manhas, M. S.; Banik, B. K.; Srirajan, V. In *The Amide Linkage: Selected Structural Aspects in Chemistry, Biochemistry and Material Science*; Greenberg, A., Breneman, C. M., Liebman, J. F., Eds.; Wiley-Interscience: New York, 2000; Chapter 7, pp 157–214.
14. For biological evaluation of β -lactams, see: (a) Vruthula, V.; Svensson, H. P.; Senter, P. D. *J. Med. Chem.* **1997**, 40, 2788–2792; (b) Clader, J. W.; Burnett, D. A.; Caplen, M. A.; Domalski, M. S.; Dugar, S.; Vaccaro, W.; Sher, R.; Browne, M. E.; Zhao, H.; Burrier, R. E.; Salisbury, B.; Davis, H. R. *J. Med. Chem.* **1996**, 39, 3684–3693; (c) Burnett, D. A.; Caplen, M. A.; Darris, H. R., Jr.; Burrier, R. E.; Clader, J. W. *J. Med. Chem.* **1994**, 37, 1733–1736; (d) Finke, P. E.; Shah, S. K.; Fletcher, D. S.; Ashe, B. M.; Brause, K. A.; Chandler, G. O.; Dellea, P. S.; Hand, K. M.; Maycock, A. L.; Osinga, D. G.; Underwood, D. J.; Weston, H.; Davies, P.; Doherty, J. B. *J. Med. Chem.* **1995**, 38, 2449–2462; (e) Mascaretti, O. A.; Boschetti, C. E.; Danelon, G. O.; Mata, E. G.; Roveri, O. A. *Curr. Med. Chem.* **1995**, 1, 441–470; (f) Edwards, P. D.; Bernstein, P. R. *Med. Res. Rev.* **1994**, 14, 127–194; (g) Buynak, J. D.; Rao, A. S.; Fod, G. P.; Carver, C.; Adam, G.; Geng, B.; Bachmann, B.; Shobassy, S.; Lackey, S. *J. Med. Chem.* **1997**, 40, 3423–3433.
15. Ames, B. N.; McCann, J.; Yamasaki, E. *Mutat. Res.* **1975**, 31, 347–364.
16. Maron, D. M.; Ames, B. N. *Mutat. Res.* **1983**, 113, 173–215.
17. (a) Ohi, R.; Gould, K. L. *Curr. Opin. Cell Biol.* **1999**, 11, 267–273; (b) Sausville, E. A.; Zaharevitz, D.; Gussio, R.; Meijer, L.; Louarn-Leost, M.; Kunick, C.; Schultz, R.; Lahusen, T.; Headlee, D.; Stinson, S.; Arbut, S. G.; Senderowicz, A. *Pharmacol. Ther.* **1999**, 82, 285–292; (c) Shah, M. A.; Schwartz, G. K. In *Cell Cycle-Mediated Drug Resistance: An Emerging Concept in Cancer Therapy*. 2001; pp 2168–2181; (d) Kumar, A. P.; Garcia, G.; Slaga, T. J. *Mol. Carcinog.* **2001**, 31, 111–124.