Chemistry of Heterocyclic Compounds, Vol. 50, No. 2, May, 2014 (Russian Original Vol. 50, No. 2, February, 2014)

4-AMINO-2-ARYL-3-CYANO-1,2-DIHYDROPYRIMIDO-[1,2-*a*]BENZIMIDAZOLES AND THEIR PYRIMIDINE ANALOGS AS NEW ANTICANCER AGENTS

V. A. Risley[1], S. Henry[1], M. V. Kosyrikhina[2], M. R. Manzanares[2], I. Payan[2], C. D. Downer[2], C. C. Hellmann[2], S. Van Slambrouck[2], and L. V. Frolova[1]*

A multicomponent condensation between 2-aminobenzimidazole, malononitrile, and an aryl or heteroaryl aldehyde was used for the synthesis of 4-amino-2-aryl-3-cyano-1,2-dihydropyrimido-[1,2-a]benzimidazoles. In addition, a new method of synthesis of the corresponding pyrimido-[2,1-a]benzimidazole derivatives was developed. These syntheses were used to prepare a library of 4-amino-2-aryl-3-cyano-1,2-dihydropyrimido[1,2-a]benzimidazoles and their corresponding pyrimido-[2,1-a]benzimidazole derivatives. This library of compounds was then tested against pancreatic and breast cancer cell lines. A number of compounds were found to possess notable anticancer activity.

Keywords: benzimidazole, 1,2-dihydropyrimidine, 1,2-dihydropyrimido[1,2-*a*]benzimidazoles, pyrimidine, pyrimido[2,1-*a*]benzimidazoles, anticancer activity, multicomponent reactions.

Multicomponent reactions (MCRs) are significant and important synthetic tools, which refer to a chemical reaction where three or more components react to form a single product [1, 2]. MCRs have become popular in modern synthetic chemistry due to their efficiency and convenience in the construction of multiple new bonds in a one-pot process and are especially useful for the synthesis of heterocyclic compounds [3].

Heterocycles are found throughout medicinal chemistry, with examples including natural and synthetic drugs such as quinine, atropine, morphine, diazepam, barbiturates, and antipyrine. Many biologically active molecules, including those previously mentioned, typically feature five- and six-membered rings containing one or more nitrogen atoms. Such privileged medicinal scaffolds, benzimidazole and pyrimidine heterocycles being perfect examples, are frequently used in the development of new drugs. These heterocycles play the roles of key pharmacophores in many different drugs expressing anticancer and antibacterial activity. It is noteworthy that the pyrimidine core is a structural constituent of critically important anticancer drugs like fluorouracil, tegafur, methotrexate, and cytabine [4]. Likewise, compounds based on the benzimidazole core are used in anticancer therapy alone, or in the mix with other drugs [5-7].

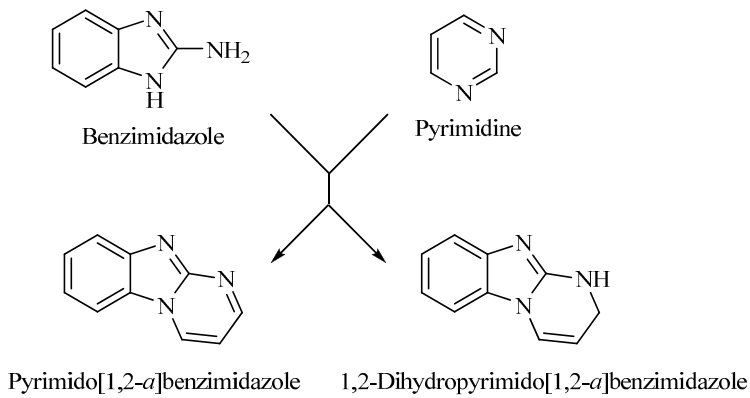
*To whom correspondence should be addressed, e-mail: frolova@nmt.edu.

[1]New Mexico Institute of Mining and Technology, 801 Leroy Place, Socorro NM 87801, U.S.A.
[2]School of Science, St. Thomas University, 16401 NW 37th Ave. Miami Gardens, FL 33054, U.S.A.; e-mail: svanslambrouck@stu.edu.

Published in Khimiya Geterotsiklicheskikh Soedinenii, No. 2, pp. 209-218, February, 2014. Original article submitted January 14, 2014.

One of the most rapidly developing areas in modern medicinal chemistry is the design of new medicinal agents using a combination of different pharmacophores in one molecule [4, 8]. Such hybrid agents could offer several potential advantages over traditional drug combinations, such as improvement of activity against multidrug resistant cell lines, improvement of pharmacokinetic profile, and synergistic activity between the pharmacophores. There are two different approaches for the design of hybrid molecules: 1) merging two different pharmocophoric moieties; 2) linking of two or more pharmacophores together [9]. The first approach is based on the ability to combine pharmacophoric groups in a new molecular structure and to retain their affinity for the biological targets. A fusion of benzimidazole and pyrimidine cycles can be a good opportunity for creating a new scaffold with anticancer properties (Scheme 1).

Scheme 1



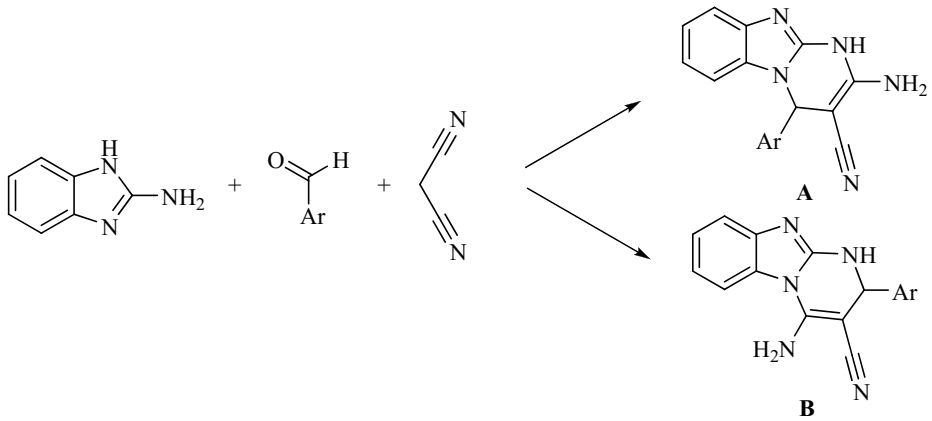
Fusion scaffolds based on benzimidazole and pyrimidine

Previously, some compounds based on the 1,2-dihydropyrimido[1,2-*a*]benzimidazole core have demonstrated antiproliferative activity [10]. These compounds differ in the mechanism of action based on the substitution pattern in the dihydropyrimidine ring. Some of them act as topoisomerase I inhibitors [11, 12], while others act as dual KSP and Aurora-A kinase inhibitors [13]. On the other hand, biological data on the activity of the pyrimido[1,2-*a*]benzimidiazole core are scarcely available in the literature, probably due to the lack of convenient methods of synthesis of core responding derivatives [14]. Interestingly, previous work on pyridine and dihydropyridine derivatives that inhibit topoisomerase II has shown that pyridine derivatives could be obtained by intracellular oxidation of dihydropyridine prodrugs [15]. In that case, corresponding pairs of compounds from both series had the same magnitude of antiproliferative activity. If our title compounds have similar mechanisms of action, the corresponding 1,2-dihydropyrimidine and pyrimidine derivatives should show similar degree of activity. For this reason, we decided to investigate systematically the antiproliferative activity of compound pairs containing 1,2-dihydropyrimido[1,2-*a*]benzimidazole and pyrimido[1,2-*a*]benz-imidazole cores.

The three-component condensation reaction of malononitrile, aromatic aldehyde, and 2-amino-benzimidazole was used to synthesize a library of 1,2-dihydropyrimido[1,2-*a*]benzimidazole derivatives. We tried several different procedures, but only the reaction in water allowed us to obtain all the desired compounds (Table 1) [16-20]. A slight modification of the procedure (time, ratio of reagents) from [16] allowed us to obtain the products **1a-k** in good yields, especially for aromatic aldehydes possessing six-membered rings. In the cases of pyrazole **1i** and thiophene **1j** derivatives, the yields were significantly lower, although it should be mentioned that we were not able to synthesize compounds bearing heteroaromatic five-membered rings using the other procedures [17-20].

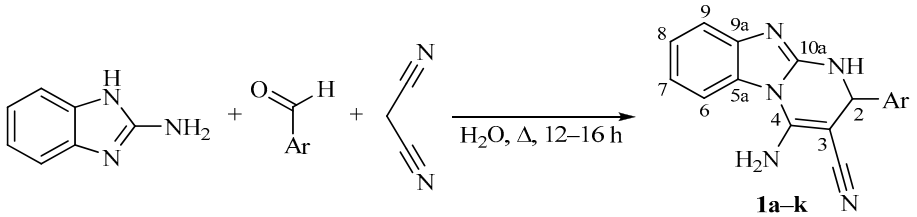
In principle, two possible heterocycles with the amino group in position 2 (structure **A**) or 4 (structure **B**) can be formed as a result of this reaction (Scheme 2). According to the literature data, the isomer **B**

Scheme 2

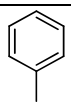
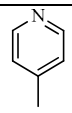
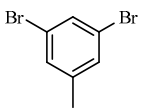
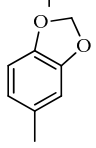
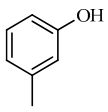
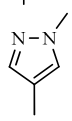
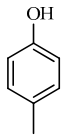
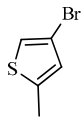
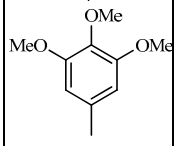
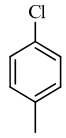
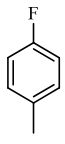


Two theoretically possible isomers of 1,2-dihydropyrimido[1,2-*a*] benzimidazole

TABLE 1. Multicomponent Synthesis of 1,2-Dihydropyrimido[1,2-*a*]-benzimidazole Derivatives **1a-k** by the Condensation Reaction of 2-Aminobenzimidazole, Malononitrile, and Aldehyde



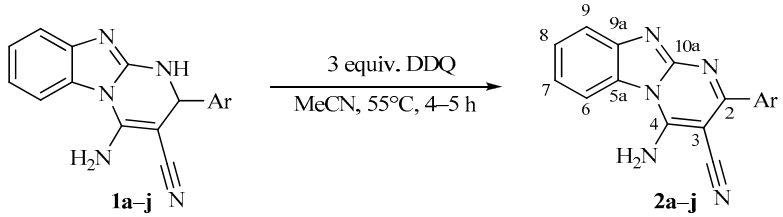
1a–k

Compound	Ar	Time, h	Yield, %	Compound	Ar	Time, h	Yield, %
1a		12	45	**1g**		14	61
1b		12	65	**1h**		15	42
1c		13	54	**1i**		12	36
1d		16	57	**1j**		14	29
1e		12	56	**1k**		12	63
1f		13	60				

is the main product of the MCR as confirmed by X-ray crystallography and by NMR spectroscopy in solution [18, 19]. Very detailed NMR investigation of compound **1k** using $^{1}H–^{13}C$-COSY and NOESY was made in [20]. Their NMR spectral data supported the existence of isomer **B** only. Unfortunately, they used a method of synthesis of compound **1k** different from the one presented here. Although the physical and spectral characteristics of compound **1k** obtained by our method and described in [20] are similar, we performed our own NOESY investigation of this compound. The presence of NOE interactions (2-CH)–H Ar and (H-6)–NH_2 and the absence of the interaction (H-6)–H Ar confirm the structure **B** for compound **1k** obtained by our method and rule out the formation of the structure **A**.

Synthesis of the corresponding pyrimido[1,2-*a*]benzimidazole derivatives **2a-j** was not straightforward (Table 2). Known procedures to obtain this type of compounds in a one-pot procedure or in a stepwise manner gave very low yields or did not work at all for many derivatives [17, 20]. Earlier, we successfully used DDQ as an oxidation reagent in the synthesis of pyrroles from pyrrolines [21]. Based on our previous experience, we tried DDQ again as an oxidizing reagent for the synthesis of pyrimido[1,2-*a*]benzimidazoles **2a-j** from the corresponding 1,2-dihydropyrimido[1,2-*a*]benzimidazole derivatives **1a-j**. 1,2-Dihydropyrimido[1,2-*a*]benzimidazole derivatives were heated at 55°C with 3 equiv. of DDQ for 4 h in acetonitrile. These reactions worked for compounds with both aromatic and heteroaromatic substituents at position 2, and the products were obtained with very high yields. The structures of new derivatives were confirmed by NMR and mass spectroscopy.

TABLE 2. Synthesis of Pyrimido[1,2-*a*]benzimidazole Derivatives **2a-j**



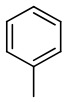
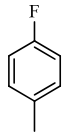
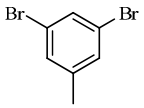
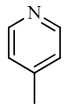
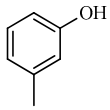
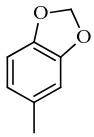
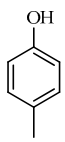
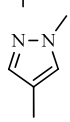
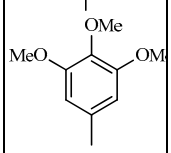
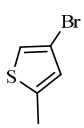
Compound	Ar	Time, h	Yield, %	Compound	Ar	Time, h	Yield, %
2a		4	41	**2f**		5	94
2b		5	87	**2g**		4	86
2c		5	54	**2h**		4	64
2d		5	73	**2i**		4	96
2e		4	84	**2j**		4	82

TABLE 3. Antiproliferative Activity of Compounds **1a-j** and **2a-j** against Human Cancer Cell Lines

Compound	IC_{50}, μM*		
	Hs578T	PANC-1	MCF-7
1a	37.5±3.5	6.25±2.5	>50
2a	39.5±0.7	48.5±3.5	>50
1b	5±1.4	18±4.2	47.3±6.4
2b	7.5±3.53	21±0	11.85±2.6
1c	9.5±0.7	5.5±1.4	>50
2c	46.25±2.5	19.3±5.1	43.5±4.9
1d	5±0.7	21.3±1.7	>50
2d	3.2±1.6	38.5±0.2	>50
1e	10±1.4	42±3.5	>50
2e	8.25±0.353	10±0	>50
1f	>50	>50	>50
2f	7.7±0.7	20.8±5.8	>50
1g	>50	>50	>50
2g	ND*[2]	ND	ND
1h	44.1±7.2	> 50	>50
2h	38.75±15.2	> 50	>50
1i	>50	> 50	>50
2i	>50	> 50	>50
1j	ND	ND	ND
2j	>50	>50	>50
1k	ND	ND	ND

*Concentration required to reduce the viability of cells by 50% after a 48 h treatment with the indicated compounds relative to a DMSO control with standard deviation from two independent experiments, each performed in four replicates, as determined by the MTT assay.
*[2]Not determined.

The obtained compounds **1a-j** and **2a-j** were tested *in vitro* against human pancreatic and breast cancer cell lines PANC-1, MCF-7, and Hs578T. Many of these compounds showed micromolar antiproliferative activity (Table 3) at varying levels, depending on the cancer cell lines and structures of the compounds used. Activities had a tendency to vary, as in some cases the 1,2-dihydropyrimido[1,2-*a*]benzimidazole derivatives were slightly more active than their respective oxidized counterparts, whereas in other cases the 1,2-dihydropyrimido[1,2-*a*]benzimidazole derivatives were either less active or had similar activity as their oxidized counterparts.

At the same time, a much stronger variation of antiproliferative activity was observed depending on the 2-aryl substituent. In general, homoaryl derivatives (compounds **1a-f**,**h**, **2a-f**,**h**) demonstrated higher activity than heteroaryl derivatives (compounds **1g-j**, **2g-j**). In addition to this, the presence of lipophilic substituents or hydroxyl groups in the *meta* position of the 2-phenyl ring generally improved the activity. Studies of SAR and possible mechanisms of action of synthesized compounds are under way.

In conclusion, we have developed a new method for the synthesis of pyrimido[1,2-*a*]benzimidazole derivatives. New 2-aryl- and 2-heteroaryl-1,2-dihydropyrimido[1,2-*a*]benzimidazoles and their oxidized analogs were synthesized and tested against several different cancer cell lines. Some pairs demonstrated low micromolar antiproliferative activity. Possible mechanisms of action will be investigated.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III 400 spectrometer (400 and 100 MHz, respectively) in DMSO-d_6, internal standard – TMS. High-resolution ESI+ mass spectra with positive ion-mode electrospray ionization (ESI+) were recorded at the Mass Spectrometry Facility, University of New Mexico, on a LCT Premier TOF mass spectrometer (compounds **1b**-**e**, **2b**,**e**) and at the Department of Chemistry and Biochemistry, Texas State University, on a Waters Synapt G2 LCMS (other compounds). Melting points were measured on a Mel-Temp Model 1001D apparatus and were uncorrected. All reactions were performed in oven-dried flasks open to the atmosphere or under nitrogen and monitored by TLC on precoated (250 μm) silica gel 60 F254 glass-backed plates (Sorbent Technologies). Visualization was accomplished with UV light. Flash column chromatography was performed on silica gel (32-63 μm, 60 Å pore size). All reagents and solvents were purchased from commercial sources (Acros Organics and Sigma-Aldrich) and used without purification.

4-Amino-2-(phenyl)-1,2-dihydropyrimido[1,2-*a*]benzimidazole-3-carbonitrile (1a). A mixture of malononitrile (0.0330 g, 0.499 mmol), 2-aminobenzimidazole (0.0666 g, 0.500 mmol), and benzaldehyde (0.0531 g, 0.500 mmol) was refluxed in water (3 ml) for 12 h. The product was separated by filtration, and the precipitate was washed with EtOH and diethyl ether. Yield 0.0650 g (45%). White powder. Mp 264-266°C (mp 267-269°C [20]). The ^{1}H NMR spectrum corresponds to the published one [20].

4-Amino-2-(3,5-dibromophenyl)-1,2-dihydropyrimido[1,2-*a*]benzimidazole-3-carbonitrile (1b) was obtained analogously from malononitrile (0.0343 g, 0.519 mmol), 2-amino benzimidazole (0.0660 g, 0.496 mmol), and 3,5-dibromobenzaldehyde (0.1002 g, 0.380 mmol). Yield 0.1445g (65%). White powder. Mp 246-247°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 5.35 (1H, s, CH); 6.96 (2H, s, NH_2); 7.02 (1H, t, *J* = 7.2, H Ar); 7.13 (1H, t, *J* = 7.2, H Ar); 7.25 (1H, d, *J* = 7.5, H Ar); 7.50 (2H, s, H Ar); 7.65 (1H, d, *J* = 7.2, H Ar); 7.79 (1H, s, H Ar); 8.66 (1H, s, NH). ^{13}C NMR spectrum, δ, ppm: 52.0 (C-2); 60.3 (C-3); 112.5 (C-6); 116.2 (C-9); 118.8 (CN); 120.0 (C-7); 122.7 (C-3,5 Ar); 123.5 (C-8); 128.3 (C Ar); 129.2 (C-5a); 132.8 (C Ar); 143.4 (C-9a); 147.4 (C Ar); 149.5 (C-10a); 151.2 (C-4). Found, *m*/*z*: 443.9443 $[M+H]^+$. $C_{17}H_{12}Br_2N_5$. Calculated, *m*/*z*: 443.9459.

4-Amino-2-(3-hydroxyphenyl)-1,2-dihydropyrimido[1,2-*a*]benzimidazole-3-carbonitrile (1c) was obtained analogously from malononitrile (0.0327 g, 0.495 mmol), 2-aminobenzimidazole (0.0664 g, 0.498 mmol), and 3-hydroxybenzaldehyde (0.0659 g, 0.540 mmol). Yield 0.0823 g (54%). White powder. Mp 234-235°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 5.09 (1H, s, CH); 6.68 (2H, t, *J* = 7.56, H Ar); 6.78 (2H, s, NH_2); 7.00 (1H, t, *J* = 7.6, H Ar); 7.12 (2H, s, H Ar); 7.23 (1H, d, *J* = 7.7, H Ar); 7.63 (1H, d, *J* = 7.5, H Ar); 8.53 (1H, s, H Ar); 9.46 (1H, s, NH). ^{13}C NMR spectrum, δ, ppm: 53.2 (C-2); 62.0 (C-3); 112.4 (C-6); 112.5 (C Ar), 114.7 (C Ar), 116.0 (C Ar), 116.4 (C-9); 119.2 (CN), 119.8 (C-7); 123.3 (C-8); 129.3 (C-5a), 129.6 (C Ar), 143.6 (C-9a), 144.5 (C Ar), 148.9 (C-4); 151.7 (C-10a); 157.5 (C–OH). Found, *m*/*z*: 304.1202 $[M+H]^+$. $C_{17}H_{14}N_5O$. Calculated, *m*/*z*: 304.1198.

4-Amino-2-(4-hydroxyphenyl)-1,2-dihydropyrimido[1,2-*a*]benzimidazole-3-carbonitrile (1d) was obtained analogously from malononitrile (0.0336 g, 0.508 mmol), 2-aminobenzimidazole (0.0658 g, 0.494 mmol), and 4-hydroxybenzaldehyde (0.0658 g, 0.516 mmol). Yield 0.0847g (57%). White powder. Mp 234-235°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 5.08 (1H, s, CH); 6.71–6.73 (3H, m, NH_2, H Ar); 6.99 (1H, t, *J* = 7.8, H Ar); 7.06-7.12 (3H, m, H Ar); 7.21 (1H, d, *J* = 7.7, H Ar); 7.62 (1H, d, *J* = 8.1, H Ar); 8.43 (1H, s, H Ar); 9.42 (1H, s, NH). ^{13}C NMR spectrum, δ, ppm: 52.9 (C-2); 62.6 (C-3); 115.0 (C-6); 115.3 (C Ar); 115.9 (C-9); 119.2 (CN); 119.7 (C-7); 123.2 (C-8); 127.3 (C Ar); 129.3 (C-5a); 133.1 (C Ar); 143.6 (C-9a); 148.9 (C-4); 151.7 (C-10a); 157.1 (C Ar). Found, *m*/*z*: 304.1202 $[M+H]^+$. $C_{17}H_{14}N_5O$. Calculated, *m*/*z*: 304.1198.

4-Amino-2-(3,4,5-trimethoxyphenyl)-1,2-dihydropyrimido[1,2-*a*]benzimidazole-3-carbonitrile *(*1e) was obtained analogously from malononitrile (0.0331 g, 0.501 mmol), 2-aminobenzimidazole (0.0661 g, 0.496 mmol), and 3,4,5-trimethoxybenzaldehyde (0.0962 g, 0.490 mmol). Yield 0.1887 g (56%). White powder. Mp 215-216°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 3.70 (9H, s, $3CH_3$); 5.18 (1H, s, CH); 6.63 (2H, s, H Ar); 6.84 (2H, s, NH_2); 7.00 (1H, t, *J* = 7.8, H Ar); 7.11 (1H, t, *J* = 7.6, H Ar); 7.23 (1H, d, *J* = 7.2, H Ar); 7.64 (1H, d, *J* = 8.0, H Ar); 8.50 (1H, s, NH). ^{13}C NMR spectrum, δ, ppm: 53.2 (C-2); 55.8 (OCH_3); 59.9 (OCH_3); 61.7 (C-3); 103.6 (C Ar);

112.2 (C-6); 116.0 (C-9); 119.0 (CN); 119.8 (C-7); 123.2 (C-8); 129.2 (C-5a); 137.1 (C Ar); 138.0 (C Ar); 143.5 (C-9a); 149.1 (C-4); 151.6 (C-10a); 152.8 (C Ar). Found, *m*/*z*: 378.1560 $[M+H]^+$. $C_{20}H_{20}N_5O_3$. Calculated, *m*/*z*: 378.1566.

4-Amino-2-(4-fluorophenyl)-1,2-dihydropyrimido[1,2-*a*]benzimidazole-3-carbonitrile (1f) was obtained analogously from malononitrile (0.0332 g, 0.502 mmol), 2-aminobenzimidazole (0.0669 g, 0.502 mmol), and 4-fluorobenzaldehyde (0.0654 g, 0.526 mmol). Yield 0.0919 g (60%). White powder. Mp 233-235°C (mp 232°C [22]). The ^{1}H NMR spectrum corresponds to the published one [22].

4-Amino-2-(4-pyridinyl)-1,2-dihydropyrimido[1,2-*a*]benzimidazole-3-carbonitrile (1g) was obtained analogously from malononitrile (0.0334 g, 0.506 mmol), 2-aminobenzimidazole (0.0669 g, 0.502 mmol), and 4-pyridinecarbaldehyde (0.0577 g, 0.539 mmol). Yield 0.0885 g (61%). Tan powder. Mp 232-235°C (mp 236°C (decomp.) [23]). The ^{1}H NMR spectrum corresponds to the published one [23].

4-Amino-2-(1,3-benzodioxolan-5-yl)-1,2-dihydropyrimido[1,2-*a*]benzimidazole-3-carbonitrile (1h) was obtained analogously from malononitrile (0.0331 g, 0.501 mmol), 2-aminobenzimidazole (0.0666 g, 0.500 mmol), and piperonal (0.0752 g, 0.501 mmol). Yield 0.0698 g (42%). Yellow powder. Mp 266-267°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 5.29 (1H, s, CH); 5.94 (2H, s, CH_2); 6.66 (1H, d, *J* = 7.2, H Ar); 6.79-6.89 (3H, m, NH_2, H Ar); 7.01 (2H, t, *J* = 7.3, H Ar); 7.13 (2H, t, *J* = 7.7, H Ar); 7.23 (1H, d, *J* = 7.7, H Ar); 8.44 (1H, s, NH). ^{13}C NMR spectrum, δ, ppm: 49.1 (C-2); 60.7 (C-3); 101.0 (CH_2); 108.1 (C Ar); 112.3 (C-6); 116.0 (C-9); 118.8 (CN); 119.6 (C Ar); 119.8 (C-7); 121.8 (C Ar); 123.2 (C-8); 124.2 (C Ar); 129.3 (C-5a); 143.6 (C-9a); 144.2 (C Ar); 147.3 (C Ar); 149.3 (C-4); 151.7 (C-10a). Found, *m*/*z*: 332.1145 $[M+H]^+$. $C_{18}H_{12}N_5O_2$. Calculated, *m*/*z*: 332.1147.

4-Amino-2-(1-methyl-1*H*-pyrazol-4-yl)-1,2-dihydropyrimido[1,2-*a*]benzimidazole-3-carbonitrile (1i) was obtained analogously from malononitrile (0.0333 g, 0.504 mmol), 2-aminobenzimidazole (0.0667 g, 0.501 mmol), and 1-methyl-1*H*-pyrazole-4-carbaldehyde (0.0552 g, 0.501 mmol). Yield 0.0440 g (30%). White powder. Mp 278-280°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 3.71 (3H, s, CH_3); 5.08 (1H, s, CH); 6.74 (2H, s, NH_2); 6.95 (1H, t, *J* = 7.6, H Ar); 7.07 (1H, t, *J* = 7.6, H Ar); 7.17 (1H, d, *J* = 7.0, H Ar); 7.24 (1H, s, H Ar); 7.50 (1H, s, H Ar); 7.57 (1H, d, *J* = 7.0, H Ar); 8.30 (1H, s, NH). ^{13}C NMR spectrum, δ, ppm: 45.9 (C-2); 62.7 (C-3); 112.9 (C-6); 116.5 (C-9); 119.5 (CN); 120.3 (C-7); 123.7 (C-8); 123.9 (C Ar); 128.9 (C Ar); 129.8 (C-5a); 136.9 (C Ar); 144.0 (C-9a); 149.7 (C-4); 152.4 (C-10a). Found, *m*/*z*: 292.1314 $[M+H]^+$. $C_{15}H_{14}N_7$. Calculated, *m*/*z*: 292.1311.

4-Amino-2-(4-bromothiophen-2-yl)-1,2-dihydropyrimido[1,2-*a*]benzimidazole-3-carbonitrile (1j) was obtained analogously from malononitrile (0.0327 g, 0.495 mmol), 2-aminobenzimidazole (0.0668 g, 0.502 mmol), and 4-bromo-2-thiophenecarbaldehyde (0.0957 g, 0.501 mmol). Yield 0.0499 g (27%). Brown powder. Mp 349-350°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 5.47 (1H, s, CH); 6.97-7.01 (5H, m, NH_2, H Ar); 7.08 (1H, t, *J* = 7.6, H Ar); 7.21 (1H, d, *J* = 7.2, H Ar); 7.60 (1H, d, *J* = 8.0, H Ar); 8.70 (1H, s, NH). ^{13}C NMR spectrum, δ, ppm: 49.3 (C-2); 61.7 (C-3); 108.6 (C-Br); 113.0 (C-6); 116.8 (C-9); 119.1 (C Ar); 120.7 (CN); 123.7 (C-7); 124.0 (C-8); 127.3 (C Ar); 129.6 (C-5a); 143.8 (C Ar); 149.8 (C-9a); 150.1 (C-4); 151.5 (C-10a). Found, *m*/*z*: 371.9910 $[M+H]^+$. $C_{15}H_{10}BrN_5S$. Calculated, *m*/*z*: 371.9918.

4-Amino-2-(4-chlorophenyl)-1,2-dihydropyrimido[1,2-*a*]benzimidazole-3-carbonitrile (1k) was obtained analogously from malononitrile (0.0367 g, 0.50 mmol), 2-aminobenzimidazole (0.0708 g, 0.50 mmol), and 4-chlorobenzaldehyde (0.0708 g, 0.50 mmol). Yield 0.1080 g (63%). White powder. Mp 239-240°C (mp 238°C [21]). The ^{1}H NMR spectrum corresponds to the published one [20].

4-Amino-2-(phenyl)pyrimido[1,2-*a*]benzimidazole-3-carbonitrile (2a). Compound **1a** (0.0150 g, 0.053 mmol) was treated with DDQ (0.027 g, 0.122 mmol) in acetonitrile (2 ml) under nitrogen atmosphere at 55°C for 4 h. The product was then filtered, and the precipitate was washed with EtOH. Yield 0.0062 g (41%). Yellow powder. Mp >300°C (mp 291-293°C [23]). The ^{1}H NMR spectrum corresponds to the published one [24].

4-Amino-2-(3,5-dibromophenyl)pyrimido[1,2-*a*]benzimidazole-3-carbonitrile (2b) was prepared analogously from compound **1b** (0.0303 g, 0.068 mmol) and DDQ (0.0543 g, 0.239 mmol). Yield 0.0265 g

(87%). White powder. Mp >300°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.44 (1H, t, *J* = 7.5, H Ar); 7.59 (1H, t, *J* = 7.0, H Ar); 7.79 (1H, d, *J* = 7.6, H Ar); 8.06 (2H, s, H Ar); 8.11 (1H, s, H Ar); 8.62 (1H, d, *J* = 7.2, H Ar). ^{13}C NMR spectrum, δ, ppm: 94.6 (C-3); 115.6 (CN); 116.7 (C-6, C Ar); 122.6 (C-9); 126.1 (C-7); 126.5 (C-8); 127.1 (C-5a); 131.0 (C Ar); 135.7 (C Ar); 141.0 (C-9a); 150.4 (C-10a); 154.8 (C-2); 161.4 (C-4). Found, *m*/*z*: 441.9310 $[M+H]^+$. $C_{17}H_{10}Br_2N_5$. Calculated, *m*/*z*: 441.9303.

4-Amino-2-(3-hydroxyphenyl)pyrimido[1,2-*a*]benzimidazole-3-carbonitrile (2c) was prepared analogously from compound **1c** (0.1088 g, 0.359 mmol) and DDQ (0.1112 g, 0.490 mmol). Yield 0.0853 g (78%). Green powder. Mp 262-264°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 6.96 (1H, d, *J* = 7.6, H Ar); 7.28-7.40 (5H, m, H Ar, NH_2); 7.54 (1H, t, *J* = 7.6, H Ar); 7.75 (1H, d, *J* = 7.3, H Ar); 8.58 (1H, d, *J* = 7.8, H Ar); 8.57 (1H, s, H Ar). ^{13}C NMR spectrum, δ, ppm: 109.4 (C-3); 115.0 (C Ar); 115.4 (C-6); 116.9 (C Ar); 117.5 (CN); 118.0 (C-9); 119.3 (C Ar); 121.5 (C-7,8); 126.0 (C Ar); 126.5 (C Ar); 129.3 (C-5a); 138.4 (C-9a); 150.6 (C-10a); 154.6 (C-OH); 157.1 (C-2); 163.6 (C-4). Found, *m*/*z*: 302.1037 $[M+H]^+$. $C_{17}H_{12}N_5O$. Calculated, *m*/*z*: 302.1042.

4-Amino-2-(4-hydroxyphenyl)pyrimido[1,2-*a*]benzimidazole-3-carbonitrile (2d) was prepared analogously from compound **1d** (0.0322 g, 0.106 mmol) and DDQ (0.0541 g, 0.238 mmol). Yield 0.0234 g (73%). Green powder. Mp >300°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.43-7.49 (5H, m, H Ar, NH_2); 7.62 (1H, t, *J* = 7.6, H Ar); 7.78 (1H, d, *J* = 7.9, H Ar); 7.97-8.01 (2H, m, H Ar); 8.64 (1H, d, *J* = 8.4, H Ar). ^{13}C NMR spectrum, δ, ppm: 76.8 (C-3); 115.2 (C-6); 115.7 (CN); 116.2 (C Ar); 116.7 (C-9); 122.4 (C-7); 125.8 (C-8); 126.9 (C Ar); 131.2 (C Ar); 131.4 (C-5a); 133.0 (C-9a); 154.4 (C-10a); 162.4 (C-OH); 163.7 (C-2); 164.9 (C-4). Found, *m*/*z*: 302.1045 $[M+H]^+$. $C_{17}H_{12}N_5O$. Calculated, *m*/*z*: 302.1042.

4-Amino-2-(3,4,5-trimethoxyphenyl)pyrimido[1,2-*a*]benzimidazole-3-carbonitrile (2e) was prepared analogously from compound **1e** (0.0303 g, 0.080 mmol) and DDQ (0.0543 g, 0.238 mmol). Yield 0.0265 g (87%). White powder. Mp >300°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 3.78 (3H, s, CH_3); 3.86 (6H, s, $2CH_3$); 7.29 (4H, m, H Ar, NH_2); 7.51 (1H, t, *J* = 7.3, H Ar); 7.66 (1H, t, *J* = 7.3, H Ar); 7.76 (1H, d, *J* = 7.2, H Ar); 8.66 (1H, d, *J* = 7.6, H Ar). ^{13}C NMR spectrum, δ, ppm: 56.7 (OCH_3); 60.8 (OCH_3); 71.2 (C-3); 107.2 (C Ar); 116.0 (C-6); 116.4 (CN); 119.2 (C-9); 123.5 (C-7); 123.7 (C-8); 128.1 (C-5a); 131.4 (C Ar); 137.2 (C-9a); 140.8 (C-10a); 153.1(C Ar); 154.9 (C-2); 163.1 (C-4). Found, *m*/*z*: 376.1397 $[M+H]^+$. $C_{20}H_{18}N_5O_3$. Calculated, *m*/*z*: 376.1398.

4-Amino-2-(4-fluorophenyl)pyrimido[1,2-*a*]benzimidazole-3-carbonitrile (2f) was prepared analogously from compound **1f** (0.0307 g, 0.101 mmol) and DDQ (0.0542 g, 0.239 mmol). Yield 0.0289 g (94%). White powder. Mp 235-236°C (mp 235-237°C [23]). The ^{1}H NMR spectrum corresponds to the published one [24].

4-Amino-2-(4-pyridinyl)pyrimido[1,2-*a*]benzimidazole-3-carbonitrile (2g) was prepared analogously from compound **1g** (0.0332 g, 0.185 mmol) and DDQ (0.0793 g, 0.349 mmol). Yield 0.0320 g (98%). Brown powder. Mp 271-272°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.41 (1H, t, *J* = 7.8, H Ar); 7.56 (1H, t, *J* = 7.4, H Ar); 7.76 (1H, d, *J* = 7.8, H Ar); 7.83 (2H, d, *J* = 5.8, H Ar); 8.60 (1H, d, *J* = 7.9, H Ar); 8.80 (2H, d, *J* = 5.2, H Ar). ^{13}C NMR spectrum, δ, ppm: 102.1 (C-3); 114.2 (C-6); 115.7 (CN); 116.4 (C-8); 117.9 (C-9); 122.9 (C Ar); 123.6 (C-7); 127.3 (C Ar); 129.7 (C-5a); 145.2 (C-9a); 150.0 (C Ar); 151.3 (C-10a); 154.9 (C-2); 162.5 (C-4). Found, *m*/*z*: 287.1045 $[M+H]^+$. $C_{16}H_{11}N_6$. Calcu lated, *m*/*z*: 287.1045.

4-Amino-2-(1,3-benzodioxolan-5-yl)pyrimido[1,2-*a*]benzimidazole-3-carbonitrile (2h) was prepared analogously from compound **1h** (0.0309 g, 0.064 mmol) and DDQ (0.0522 g, 0.230 mmol). Yield 0.0197 g (64%). Green powder. Mp 235-236°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 6.18 (2H, s, CH_2); 7.14 (1H, d, *J* = 7.8, H Ar); 7.20 (2H, d, *J* = 7.9, H Ar); 7.60 (2H, t, *J* = 7.4, H Ar); 7.76 (1H, d, *J* = 7.8, H Ar); 8.37 (2H, s, NH_2); 8.61 (1H, d, *J* = 7.7, H Ar). ^{13}C NMR spectrum, δ, ppm: 78.0 (C-3); 102.4 (CH_2); 103.4 (C-6); 108.2 (C Ar); 109.2 (C Ar); 109.7 (C Ar); 114.3 (C Ar); 115.1 (CN); 124.3 (C-9a); 126.1 (C Ar); 130.3 (C-5a); 147.2 (C Ar); 148.8 (C-10a); 150.1 (C Ar); 153.5 (C-2); 161.0 (C-4). Found, *m*/*z*: 330.0911 $[M+H]^+$. $C_{18}H_{12}N_5O_2$. Calculated, *m*/*z*: 330.0911.

4-Amino-2-(1-methyl-1*H*-pyrazol-4-yl)pyrimido[1,2-*a*]benzimidazole-3-carbonitrile (2i) was prepared analogously from compound **1i** (0.0273 g, 0.094 mmol) and DDQ (0.0547 g, 0.241 mmol). Yield 0.0265 g

(97%). Olive green powder. Mp >400°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 3.97 (3H, s, CH_3); 7.48 (1H, t, *J* = 7.8, H Ar); 7.62 (1H, t, *J* = 7.6, H Ar); 7.68 (1H, d, *J* = 7.8, H Ar); 8.25 (1H, s, H Ar); 8.60–8.61 (2H, m, H Ar). ^{13}C NMR spectrum, δ, ppm: 71.3 (C-3); 102.0 (C-6); 114.2 (C-6); 115.7 (C Ar); 116.8 (CN); 118.4 (C-9); 119.4 (C-7); 123.1 (C-8); 126.1 (C Ar); 127.8 (C-5a); 129.7 (C Ar); 133.5 (C-9a); 140.0 (C-10a); 151.3 (C-2); 154.8 (C-4). Found, *m*/*z*: 290.1157 $[M+H]^+$. $C_{15}H_{12}N_7$. Calculated, *m*/*z*: 290.1154.

4-Amino-2-(4-bromothiophen-2-yl)pyrimido[1,2-a]benzimidazole-3-carbonitrile (2j) was prepared analogously from compound **1j** (0.0110 g, 0.030 mmol) and DDQ (0.0205 g, 0.090 mmol). Yield 0.0091 g (84%). Orange powder. Mp 363-364°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.37 (1H, t, *J* = 7.8, H Ar); 7.52 (1H, t, *J* = 7.3, H Ar); 7.69 (1H, d, *J* = 7.8, H Ar); 8.05 (1H, s, H Ar); 8.07 (1H, s, H Ar); 8.55 (1H, d, *J* = 7.8, H Ar). ^{13}C NMR spectrum, δ, ppm: 109.9 (C-3, C-Br); 115.5 (CN); 117.2 (C-6); 122.5 (C-9); 126.9 (C-7, C Ar); 127.0 (C-8, C Ar); 130.7 (C-5a); 131.8 (C-9a); 143.0 (C Ar); 150.1 (C-10a); 154.2 (C-2); 155.0 (C-4). Found, *m*/*z*: 369.9761 $[M+H]^+$. $C_{15}H_9BrN_5S$. Calculated, *m*/*z*: 369.9762.

Cell Culture. Human pancreatic and breast cancer cell lines PANC-1 (ATCC® CRL-1469TM), MCF-7 (ATCC® HTB-22TM), and Hs578T (ATCC® HTB-126TM) were obtained from the ATCC®. All cell lines were maintained on a tissue culture plastic substrate in a mixture of Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10% fetal bovine serum (Life Technologies, NY, USA), at 37°C in a humidified atmosphere containing 10% CO_2.

Determination of *in vitro* Cytotoxicity Using the MTT Colorimetric Assay. The antiproliferative effect of a library of compounds was tested in accordance with Romijn et al. [25]. Briefly, mitochondrial dehydrogenase activities were measured by MTT reagent (Sigma, MO, USA). The cells were seeded in 96-well plates at a density of 1.5×10^4 cells in 100 μl of DMEM medium and initially treated with concentrations of compounds **1a**-**k** and **2a**-**j** of 50 μM in 100 μl of medium. After 48 h incubation, 100 μl medium was removed prior to the addition of MTT reagent (5 mg/ml). Subsequently, IC_{50} values were determined for those compounds exhibiting strong inhibitory effects at 50 μM, using concentrations ranging from 0.1 to 50 μM. Three or four independent experiments were carried out in which four to eight wells were used for each concentration to determine the mean optical density, using an Epoch microplate spectrophotometer controlled with Gen5 Data Analysis software (Biotek, VT, USA).

This work was supported by National Institute of General Medical Sciences (P20GM103451) and federal and private funding, including STEM-TRAC, USDA, AREA programs, Florida Blue and Domingo Moreira.

Also the authors wish to thank Prof. Alexander Kornienko, Department of Chemistry and Biochemistry, Texas State University, and Mass Spectrometry Facility, University of New Mexico for mass spectra.

REFERENCES

1. J. E. Biggs-Houck, A. Younai, and J. T. Shaw, *Curr. Opin. Chem. Biol*., **14**, 371 (2010).
2. B. B. Shigate, *Org. Chem. Curr. Res.*, **1**, e107 (2012).
3. H. Eckert, *Molecules*, **17**, 1074 (2012).
4. T. P. Selvam, C. R. James, P. V. Dniandev, and S. K. Valzita, *Res. Pharm.*, **2**, № 3, 01 (2012).
5. S. L. Khokra and D. Choudhary, *Asian J. Biochem. Pharm. Res.*, **1**, № 3, 476 (2011).
6. C.-S. Chang, J.-H. Guh, C.-M. Teng, S.-L. Pan, W.-L. Chang, J.-F. Liu, K.-W. Chang, and S.-C. Kuo, US Pat. Appl. 20100179147A1.
7. Y. Chen, L. Yang, F. Feng, Q. Ge, D. Guo, and Y. Chen, PCT Int. Appl. WO 2013040286.
8. E. C. Breen and J. J. Walsh, *Curr. Med. Chem.*, **17**, 609 (2010).
9. S. Fortin and G. Bérubé, *Expert Opin. Drug Discov.*, **8**, 1029 (2013).

10. W. P. Nawrocka, B. Sztuba, A. Dryś, J. Wietrzyk, J. Kosendiak, and A. Opolski, *Pol. J. Chem.*, **80**, 279 (2006).
11. N. Zanatta, S. S. Amaral, A. Esteves-Souza, A. Echevarria, P. B. Brondani, D. C. Flores, H. G. Bonacorso, A. F. C. Flores, and M. A. P. Martins, *Synthesis*, 2305 (2006).
12. H.-J. Lee, J. S. Kim, M.-E. Suh, H. J. Park, S. K. Lee, H.-K. Rhee, H. J. Kim, E.-K. Seo, C. Kim, C.-O. Lee, and H.-Y. Park Choo, *Eur. J. Med. Chem.*, **42**, 168 (2007).
13. R.-g. Fu, Q.-d. You, L. Yang, W.-t. Wu, C. Jiang, and X.-l. Xu, *Bioorg. Med. Chem.*, **18**, 8035 (2010).
14. A. El-Shekeil, A. O. Obeid, and S. Al-Aghbari, *Eur. J. Chem.*, **3**, 356 (2012).
15. N. M. Evdokimov, S. Van Slambrouck, P. Heffeter, L. Tu, B. Le Calve, D. Lamoral-Theys, C. J. Hooten, P. Y. Uglinskii, S. Rogelj, R. Kiss, W. F. A. Steelant, W. Berger, J. J. Yang, C. G. Bologa, A. Kornienko, and I. V. Magedov, *J. Med. Chem.*, **54**, 2012 (2011).
16. A. Shaabani, A. Rahmati, A. H. Rezayan, M. Darvishi, Z. Bardri, and A. Sarvari, *QSAR Comb. Sci.*, **26**, N 9, 973 (2007).
17. M. D. Wendt, A. Kunzer, R. F. Henry, J. Cross, and T. G. Pagano, *Tetrahedron Lett.*, **48**, 6360 (2007).
18. A. Dandia, P. Sarawgi, A. L. Bingham, J. E. Drake, M. B. Hursthouse, M. E. Light, and R. Ratnani, *J. Chem. Res.*, 155 (2007).
19. H. M. E. Hassaneen, H. M. Hassaneen, S. F. M. Khiry, and R. M. Pagni, *Z. Naturoforsch.*, **63b**, 217 (2008).
20. B. Insuasty, A. Salcedo, R. Abonia, J. Quiroga, M. Norgueras, and A. Sanchez, *Heterocycl. Commun.*, **8**, 287 (2002).
21. L. V. Frolova, N. M. Evdokimov, K. Hayden, I. Malik, S. Rogelj, A. Kornienko, and I. V. Magedov, *Org. Lett.*, **13**, 1118 (2011).
22. S. A. Komykhov, K. S. Ostras, A. R. Kostanyan, S. M. Desenko, V. D. Orlov, and H. Meier, *J. Heterocycl. Chem.*, **42**, 1111 (2005).
23. A. R. Karimi and F. Bayat, *Lett. Org. Chem.*, **8**, 631 (2011).
24. A. B. A. El-Gazzar, *Egypt. J. Chem.*, **45**, 995 (2002).
25. J. C. Romijn, C. F. Verkoelen, and F. H. Schroeder, *Prostate*, **12**, 99 (1988).