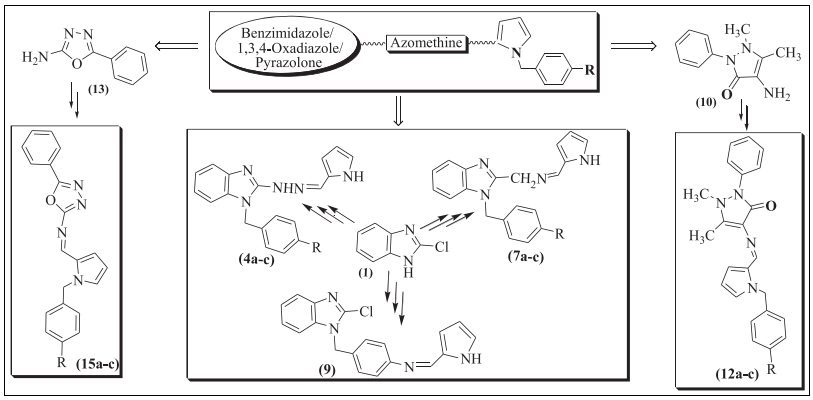
Synthesis and Cytotoxic Evaluation of Pyrrole Hetarylazoles Containing Benzimidazole/Pyrazolone/1,3,4-Oxadiazole Motifs

Bereket Mochona,[a]* Timothy Jackson,[a] DeCoria McCauley,[a] Elizabeth Mazzio,[b] and Kinfe K. Redda[b]

[a]Department of Chemistry, College of Science and Technology, Florida A&M University, Tallahassee 32307, FL, USA
[b]College of Pharmacy and Pharmaceutical Sciences, Florida A&M University, Tallahassee 32307, FL, USA
*E-mail: bereket.mochona@famu.edu

Received December 28, 2014
DOI 10.1002/jhet.2501
Published online 00 Month 2015 in Wiley Online Library (wileyonlinelibrary.com).



Azomethine-linked pyrrole bishetarylazoles containing benzimidazole/pyrazolone/1,3,4-oxadiazole were synthesized in satisfactory yields. Their structures were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, and elemental analysis. Evaluation for the cytotoxic activities *in vitro* against a panel of breast cancer cell lines (MDA-AB-231, BT-474, and Ishikawa cells) revealed that the pyrrole–benzimidazole hybrids are more potent than the pyrazolone and 1,3,4-oxadiazole hybrids in all cell lines. Compound **9** displayed promising cytotoxicity against BT-474 cell line with IC_{50} values, 7.7 μM.

J. Heterocyclic Chem., **00,** 00 (2015).

INTRODUCTION

Hetarylazoles form by far the largest class of heterocyclic compounds and are of immense importance biologically and industrially. Owing to their versatile chemotherapeutic activities, safety profiles, and high therapeutic indices, a significant amount of research effort has been directed towards combining hetarylazoles to investigate additive biological activities [1–9]. Benzimidazoles, their aryl, and alkyl-substituted derivatives have evoked considerable attention as members of hetarylazoles with antifungal, antioxidant, antihypertensive, cardiotonic, antithrombotic, antiulcer, antitubercular, antitumor activity against several tumor cell lines and so on [10–12].

On the other hand, five-membered 1,3,4-oxadiazole heterocyclics are associated with a variety of pharmacological actions and serve as intermediates for the development of bioactive molecules. Molecular modeling and pharmacokinetic studies have also demonstrated that incorporating the 1,3,4-oxadiazole moiety in drug-like molecules changes polarity, flexibility as well as metabolic profiles, and the ability to engage in hydrogen-bonding interaction with receptors. Hence, 1,3,4-oxadiazoles have been widely employed as isosteric substituents for esters and amides in a number of biological targets [13–18]. The pyrazolone derivative, 4-aminoantipyrine, is a strong inhibitor of cyclooxygenase isoenzymes, platelet thromboxane synthesis, and prostanoids synthesis. The biological activity of the 4-aminoantipyrine has been attributed to its scavenging activity against reactive oxygen and nitrogen species, as well as to the inhibition of neutrophils' oxidative burst [19–26].

A recent survey of novel small-molecule therapeutics has revealed that the majority of the drugs result from an analog-based approach and that their market share represents two-thirds of all drug sales. Hence, combination of two pharmacophores into a single molecule or molecular hybridization represents an important part of the efforts to overcome drug resistance in anticancer and antimicrobial agents search. The reported individual/chemical/pharmacological properties of benzimidazole, 1,3,4-oxadiazole, and pyrazolone functionalities compelled us to design and synthesize hetarylazole molecule bearing strategic hybrid combinations of the aforementioned moieties and investigate the anticancer activities of these novel agents [27–31]. Herein, we report the synthesis and preliminary anticancer evaluation of bishetarylazole hybrids bearing pyrrole moieties and benzimidazole/4-aminoantipyrine/1,3,4-oxadiazole nucleus.

RESULTS AND DISCUSSION

Chemistry. The synthetic strategies adopted for the synthesis of the intermediates and target compounds are

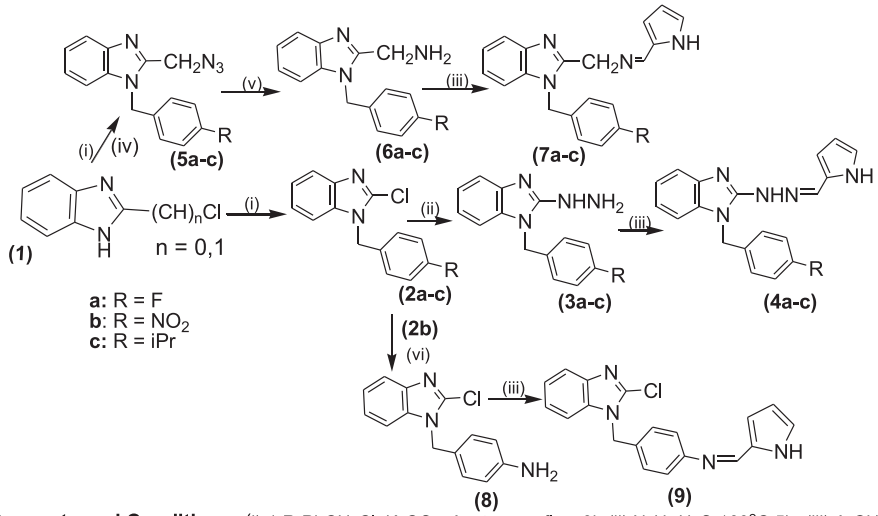
depicted in Schemes 1, 2, and 3. In Scheme 1, 1-(4-substituted benzyl)-2-chlorobenzimidazoles, **2a–c** were prepared from the commercially available 2-chlorobenzimidazole under basic condition. Treatment of **2** with hydrazine hydrate resulted in the benzimidazol-2-hydrazine derivatives **3a–c**. The physical properties of compounds **2** and **3** are summarized in Table 1. The reaction of compound **3** with 2-pyrrole carboxaldehyde under acidic condition afforded the targeted bishetaryls of benzimidazole and pyrrole (**4a–c**) in good to excellent yields. The physical and analytical data of compounds **4a–c** are outlined in Table 2. Similarly, 2-azidomethylbenzimidazole (5) obtained from **1** was reduced to afford benzimidazol methanamine intermediates **6a–c**. Condensation of **6** with pyrrole-2-carboxaldehyde resulted in **7a–c** in good yields. Compound **9** was prepared in two steps from **2b**, that is, reduction of nitro group followed by condensation with an aldehyde. The ^{1}H-NMR spectra of hydrazines (**4**) indicate multiplet aromatic protons at 6.9–7.4 ppm and the signals of the pyrrolyl ring protons at 6.0, 6.3, and 6.7 ppm. The azomethine and methylene protons appear at 7.4 and 5.2 ppm, respectively. The ^{13}C-NMR shows the azomethine carbon signal at 152 ppm. In the IR spectrum of **4**, the absorption band for stretching vibrations of the C=N group of the azomethine fragment is observed at 1640 cm^{-1}.

In Scheme 2, 4-aminoantipyrine (**10**) was condensed with pyrrole carboxaldehyde and afforded the expected Schiff bases in excellent yields (Table 3). In the ^{1}H-NMR spectrum of the Schiff base of 4-aminoantipyrine (**11**), the signal for azomethine proton (–CH=N–) appears as a singlet at 9.2–10.5 ppm. The pyrrole (NH) proton appears as a singlet at 11.0–11.8 ppm. The multiplet signals obtained in the δ 7.0–8.0 ppm range are due to the aromatic protons. The signal for pyrazolone ring carbon-attached methyl protons (–CH_3) appears as a singlet at δ 2.42 ppm, while that for pyrazolone ring nitrogen-attached methyl protons (=N–CH_3) appears as a singlet at δ 3.06 ppm. In the ^{13}C-NMR spectrum, the azomethine carbon signal has appeared at 152 ppm. The pyrazolone ring carbon-attached methyl carbon (–CH_3) and pyrazolone ring nitrogen-attached methyl carbon (=N–CH_3) peaks have been observed in the expected range at 8–32 ppm. The aromatic carbon signals are seen at 106–157 ppm range depending on their electronic environment. The IR spectrum of the Schiff base displays a sharp band at 1600–1630 cm^{-1}, which can be assigned to –C=N stretching frequency. Further, the Schiff base exhibits a band at 1655 cm^{-1} due to –C=O.

In Scheme 3, condensation of 2-amino-5-phenyl 1,3,4-oxadiazole **(13)** with 2-pyrrole carbaldehyde generated the Schiff base derivatives (**14**) and N-benzylated derivatives (**15**) in good yields. The three protons of pyrrole ring in targeted compounds appeared as multiplets between δ 6.27 and 7.49. The ^{1}H-NMR spectra also supported the proposed structure as there was no signal corresponding to NH_2 proton.

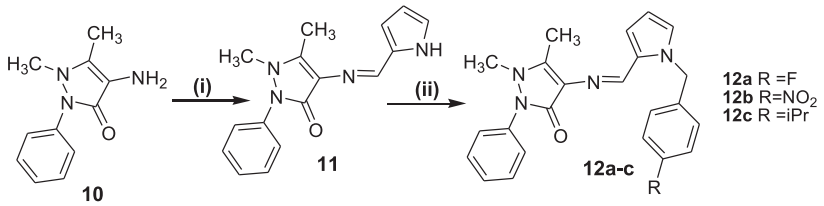
Cytotoxicity. All of the newly synthesized compounds were evaluated for their anticancer effects using breast cancer cell lines, MDA-MB-231, Ishikawa cells, and BT-474 cells. Each compound stored at 20 m*M* was diluted from 100 μ*M* to 10 μ*M* by fivefold serial dilutions. Cells were treated with each compound for 48 h, followed by measuring cell growth rates by sulforhodamine B-based spectrophotometry. The IC_{50} concentration for each compound was calculated with reference to a control sample, which represents the concentration that results in a 50% decrease in cell growth after 48 h

Scheme 1



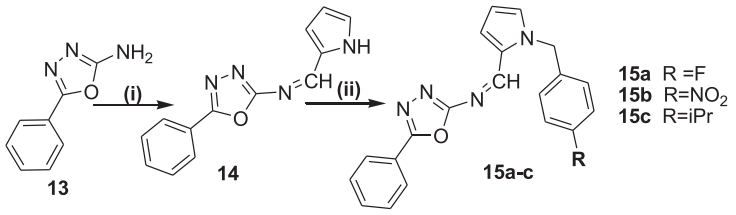
Reagents and Conditions: (i) 4-R-PhCH$_2$Cl, K_2CO_3, Acetone, reflux, 8h (ii) $N_2H_4.H_2O$ 160°C,5h (iii) ArCHO, EtOH, 60°C, 2h (iv) NaN_3, DMSO, rt-50°C, 4h (v) NH_4Cl, EtOH/H_2O, Zn, reflux, 4h (vi) 10% Pd/C, H_2, EtOH

Scheme 2



Reagents and Conditions: (i) 2-pyrrole carboxaldehyde, EtOH, AcOH (iii) NaH/DMF, $RC_6H_4CH_2X$

Scheme 3



Reagents and Conditions: (i) 2-pyrrole carboxaldehyde, EtOH, AcOH (ii) NaH/DMF, $RC_6H_4CH_2X$

incubation in the presence of the test compound, and the values are presented in Table 4. The data for pacletaxel was included as a reference.

The resultant data showed that all the synthesized compounds exhibited moderate activity against MDA-MB-231 cell lines except **15b** and **15c**. Compound **15c** was inactive in all cell lines. Compounds **4b**, **7c**, **9**, **12c**, and **15a** were active against all the three cell lines, compound **9** being the most active and compound **15c** the least from the series. The pyrrole-benzimidazole series exhibited moderate activity compared with the pyrazolone and 1,3,4-oxadiazole series.

CONCLUSIONS

Bishetarylazoles of benzimidazole, 1,3,4-oxadiazole, and pyrazolone bearing pyrrole moieties were synthesized and assessed for cytotoxicity *in vitro* against breast cancer cell lines. The benzimidazole series displayed the optimal profiles with IC_{50} in micromolar range. The most promising compound **9** with an $IC_{50} = 7.7\ \mu M$ is an attractive candidate for further assessment. This observation could be attributed to the synergetic effect that may result from combining the *N*-benzylpyrrolyl core with the typical benzimidazole core.

EXPERIMENTAL

Synthesis. Reagents and solvents were purchased from Sigma-Aldrich Chemical Company Inc. (St. Louis, Mo, USA) and used as received. The melting points (mp) were determined using Mel-Temp apparatus and were uncorrected. The infrared spectra were obtained using Perkin-Elmer 1430 FT spectrometer (USA) and are reported in inverse centimeters. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker-300 MHz spectrometer (MA, USA). Chemical shifts (in ppm) are reported relative to TMS as internal standard for solutions in DMSO-d_6 and $CDCl_3$. Column chromatography was performed using silica gel (200–425 mesh). Analytical thin-layer chromatography was performed on 250-µm-layer flexible plates. Spots were visualized under UV light. Elemental analyses for C, H, and N were within 0.4% of the calculated values.

Synthesis of 1-(4-substituted benzyl)-2-chlorobenzimidazoles (2a–c). 2-Chloro-1*H*-benzoimidazole (20 mmol) was dissolved in dry DMF (15 mL) at 0°C, to the solution was added NaH (22.7 mmol), and the mixture was stirred for 1 h at 0°C, and then halide (21.6 mmol) was added. The mixture was stirred overnight at room temperature and was poured into water (50 mL) and stirred for 1 h, filtrated, washed with water, and dried to afford **2a–c**. Yields, recrystallization solvents, and melting points of the products are reported in Table 1.

2-Chloro-1-(4-fluorobenzyl) benzimidazole (2a). White solid, IR (KBr): 1450, 1360, 720. ^{1}H-NMR ($CDCl_3$): δ 5.13 (s, 2H, CH_2), 7.10–7.49 (m, 8H, Ar-H).

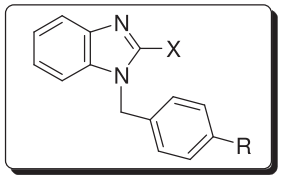
2-Chloro-1-(4-nitrobenzyl)benzimidazole (2b). White solid, IR (KBr): 1450, 1360, 720. ^{1}H-NMR ($CDCl_3$): δ 5.44 (s, 2H, CH_2), 7.10–7.49 (m, 8H, Ar-H).

2-Chloro-1-(4-isopropylbenzyl)benzimidazole (2c). White solid, IR (KBr): 1450, 1360, 720. ^{1}H-NMR ($CDCl_3$): δ 1.08 (d, 6H, –CH($\underline{CH_3}$)$_2$), 3.12 (m, 1H, $\underline{CH}(CH_3)_2$), 5.09 (s, 2H), 7.18 (d, $J = 6.5$ Hz, 2H, Ar-H), 7.23–7.34 (m, 6H, Ar-H), 7.72 (d, $J = 7.0$ Hz, 1H, Ar-H).

Synthesis of 2-benzimidazole hydrazine (3a–c). *N*-benzyl-2-chlorobenzimidazole **(2a–c)** (1 mmol) was heated at 160°C in a Pyrex capped tube with 0.1 mL of hydrazine hydrate for 5 h. After the mixture was cooled, a white solid separated, which was collected and recrystallized.

Table 1

Physical properties of *N*-benzyl-2-chlorobenzimidazoles and 2-mehanaminebenzimidazoles.



No.	X	R	Yield (%)	Recryst. solv	mp (°C)
2a	Cl	F	68	Pet. ether	121–123
2b	Cl	NO_2	64	Pet. ether	84–85
2c	Cl	iPr	70	Pet. ether	118–121
3a	$NHNH_2$	F	67	H_2O	138–141
3b	$NHNH_2$	NO_2	64	Pet. ether	84–85
3c	$NHNH_2$	iPr	88	H_2O	156–158

Yields, recrystallization solvents, and melting points of the products are reported in Table 1. Spectral data for **3c**, which is representative of the title compounds, are listed in the succeeding text.

1-(4-Isopropylbenzyl)-2-benzimidazole hydrazine (3c). IR (KBr):3320, 3225, 1550, 720. ^{1}H-NMR, (DMSO-d_6,): δ 1.08 (d, 6H, –CH $(CH_3)_2$), 6.91–7.30 (m, 4H, Ar-H).

Synthesis of the hydrazine Schiff base (4a–c). A mixture of pyrrole-2-carboxaldehyde (10 mmol) and **3a–c** (10 mmol) in methanol (10 ml) containing three drops of glacial acetic acid was heated to reflux for 1.5 h. The resulting Schiff base precipitated on cooling. The precipitate was filtered off and recrystallized from absolute ethanol.

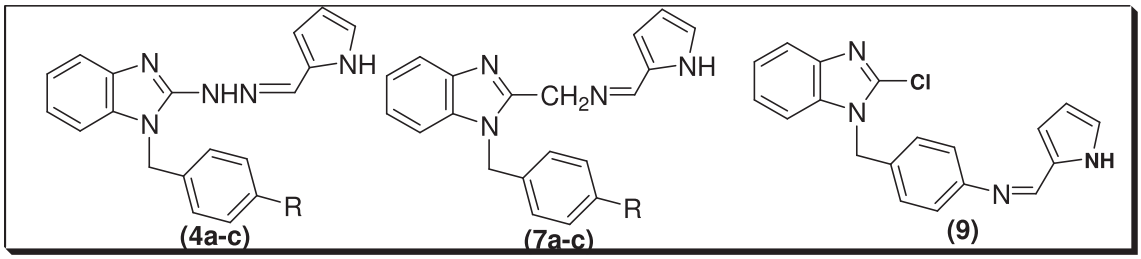
2-(2-Pyrolylmethylene)-1-(4-fluorobenzyl)-benzimidazol-2-hydrazine (4a). White solid; 72% yield; mp 162–164°C; ^{1}H-NMR ($CDCl_3$, δ, ppm): 5.58 (s, 2H, Ph–CH_2), 7.05–7.15 (m, 3H, CH-pyrrole),6.97–7.82 (m, 8H, Ar-H), 10.77 (s, 1H, CH=N). Analysis for: $C_{19}H_{16}FN_5$. Found, %: C 68.62; H 4.86; N 21.88. Calc, %: C 68.46; H 4.84; N 21.01.

2-(2-Pyrolylmethylene)-1-(4-nitrobenzyl)-benzimidazol-2-hydrazine (4b). Yellow solid, 65% yield; mp 155–158°C; IR (KBr):1660 (C=O), 1614 (C=N). ^{1}H-NMR ($CDCl_3$, δ, ppm): 5.85 (s, 2H, Ph–CH_2), 6.65–7.35 (m, 3H, CH-pyrrole), 7.30–7.90 (m, 8H, Ar-H), 10.85 (s, 1H, –CH=N). Analysis for: $C_{19}H_{16}N_6O_2$. Found, %: C 63.43; H 4.12; N 23.64. Calc, %: C 63.32; H 4.48; N 23.32.

2-(2-Pyrolylmethylene)-1-(4-isopropylbenzyl)-benzimidazol-2-hydrazine(4c). White powder, 64% yield; mp 186–188°C; IR (KBr): 1643 (C=O), 1586 (C=N). ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.08 (d, 6H, –CH($\underline{CH_3}$)$_2$), 3.12 (m, 1H, $\underline{CH}$ $(CH_3)_2$), 5.58 (s, 2H, Ph–CH_2), 6.81–7.57 (m, 3H, CH-pyrrole), 6.98–7.20 (m, 8H, Ar-H), 7.0 (1H, NH), 10.41 (s, 1H, –N=CH). Analysis for: $C_{22}H_{23}N_5$. Found, %: C 73.62; H 6.16; N 19.87. Calc, %: C 73.92; H 6.49; N 19.59.

Table 2

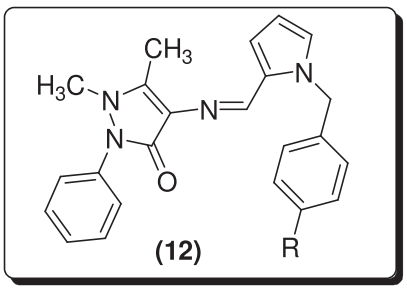
Physical and analytical data of pyrrolyl-benzimidazole hybrids.

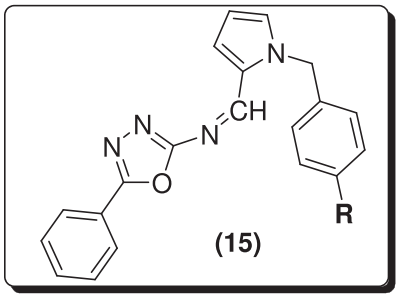


No.	Formula	MW	Yield (%)	logP	mp (°C)	C	H	N	
4a	$C_{19}H_{16}FN_5$	333.36	72	4.02	162–164	68.46	4.84	21.01	Calc
						68.62	4.86	21.88	Found
4b	$C_{19}H_{16}N_6O_2$	360.37	65	3.81	155–158	63.32	4.48	23.32	Calc
						63.43	4.12	23.64	Found
4c	$C_{22}H_{23}N_5$	357.2	64	5.37	186–188	73.92	6.49	19.59	Calc
						73.62	6.16	19.87	Found
7a	$C_{20}H_{17}FN_4$	332.37	56	3.74	165–168	72.27	5.16	16.86	Calc
						72.52	5.18	16.8	Found
7b	$C_{20}H_{17}N_5O_2$	359.38	76	3.54	168–169	66.84	4.77	19.49	Calc
						66.66	4.38	19.32	Found
7c	$C_{23}H_{24}N_4$	356.46	67	5.09	150–151	77.5	6.79	15.72	Calc
						77.96	6.89	15.22	Found
9	$C_{19}H_{15}ClN_4$	334.8	56	4.45	145–147	68.16	4.52	16.73	Calc
						68.66	4.34	16.87	Found

Table 3

Physical and analytical data of compounds 12a–c and 15a–c.





No.	Formula	MW	Yield (%)	logP	mp (°C)	C	H	N	
12a	$C_{23}H_{21}FN_4O$	388.44	56	3.83	165–168	71.12	5.45	14.42	Calc
						71.52	5.18	14.80	Found
12b	$C_{23}H_{21}N_5O_3$	415.44	65	3.63	155–158	66.49	5.09	16.86	Calc
						66.43	5.12	16.64	Found
12c	$C_{26}H_{28}N_4O$	412.53	64	5.18	186–188	75.70	6.84	13.58	Calc
						75.62	6.16	13.87	Found
15a	$C_{20}H_{15}FN_4O$	346.36	79	4.44	165–168	69.35	4.37	16.18	Calc
						69.36	4.08	16.52	Found
15b	$C_{20}H_{15}N_5O_3$	373.36	68	4.24	155–158	64.34	4.05	18.76	Calc
						64.12	4.36	18.43	Found
15c	$C_{23}H_{22}N_4O$	370.45	81	5.79	168–169	74.57	5.99	15.12	Calc
						74.38	5.41	15.06	Found

Table 4

Cytotoxicity activity on breast cancer cell lines.

	IC_{50} (μM)		
	MDA-AB-231	Ishikawa	BT-474
4a	84.1	77.2	>500
4b	72.2	67.7	53
4c	82	55	>500
7a	76.22	88.2	>500
7b	64.1	>500	>500
7c	37.8	65.2	78.41
9	23.26	9.07	7.7
12a	66.7	>500	>500
12b	49.8	>500	>500
12c	53.26	89.07	77.7
15a	84.2	78.5	68.3
15b	>500	66.9	88.1
15c	>500	>500	>500
Pacletaxel	0.003	0.003	0.005

General procedures for the synthesis of compounds 5a–c. 1-(4-Fluorobenzyl)-2-chloromethylbenzimidazole (10 mmol) was added to 11 mmol NaN_3 in DMSO solution. The mixture was heated at 50°C for 4 h. The reaction was quenched with water (10 mL), extracted with chloroform, and washed with water and brine, and the organic layer was dried over $MgSO_4$ and evaporated under reduced pressure and purified by column chromatography to afford pure azide.

1-(4-Fluorobenzyl)-2-azidomethyl-1H-benzoimidazole (5a). Yield: (67%), mp 139–141°C. ^{1}H-NMR ($CDCl_3$): δ 4.55 (s, 2H, CH_2), 7.33–7.87 (m, 8H).

General procedure for the synthesis of compounds 6a–c. To the solution of azide (15 mmol) and ammonium chloride (35 mmol) in ethyl alcohol (20 mL) and water (7 mL), zinc powder (20 mmol) was added and refluxed for 4 h. The mixture was diluted with ethylacetate, and aqueous ammonia (5 mL) was added. The mixture was filtered and washed with brine and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography.

1-(4-Fluorobenzyl)-2-benzimidazol methanamine (6a). Recrystallized from EtOAc to give 1.78 g (75% yield) of cream crystals; mp 269–271°C; ^{1}H-NMR ($CDCl_3$, δ, ppm):3.90 (s, 2H, –$\underline{CH_2}$–NH_2), 5.18 (s, 2H, Ph–CH_2), 6.97–7.82 (m, 8H, Ar-H).

1-(4-Nitrobenzyl)-2-benzimidazol methanamine (6b). Light yellow solid; 75% yield; mp 123–125°C; IR (KBr):1660 (C=O), 1614 (C=N). ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.94 (s, 2H, –$\underline{CH_2}$–NH_2), 5.30 (s, 2H, Ph–CH_2), 7.01–7.82 (m, 8H, Ar-H).

1-(4-Isopropylbenzyl)-2-benzimidazol methanamine (6c). White solid; 75% yield; mp 132–134°C; IR (KBr): 1643 (C=O), 1586 (C=N). ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.08 (d, 6H, –$CH(CH_3)_2$), 3.83 (s, 3H CH_3), 5.58 (s, 2H, Ph–CH_2), 6.98–7.20 (m, 8H, Ar-H).

General procedure for the synthesis of compounds 7a–c. 2-Pyrrole carboxaldehyde (10 mmol) and few drops of glacial acetic acid were added to a solution of 4-amino-1,

5-dimethyl-2-phenylpyrazol-3-one (10 mmol) in anhydrous ethanol (20 mL) at room temperature. The reaction mixture was refluxed for 2 h and then cooled at room temperature. The solid formed was filtered and washed with ether to provide the desired Schiff base.

N-(2-pyrolylmethylene)-1-(4-fluorobenzyl)-benzimidazol-2-methanamine (7a). White powder, 56% yield; mp 143–145°C; ^{1}H-NMR ($CDCl_3$): δ 3.90 (s, 2H, –$\underline{CH_2}$–NH_2), 5.18 (s, 2H, Ph–CH_2), 6.97–7.82 (m, 8H, Ar-H). Analysis for: $C_{20}H_{17}FN_4$. Found, %: C 72.52; H 5.18; N 16.80. Calc, %: C 72.27; H 5.16; N 16.86.

N-(2-pyrolylmethylene)-1-(4-nitrobenzyl)-benzimidazol-2-methanamine (7b). Yellow solid; 76% yield; mp 269–271°C; IR (KBr):1660 (C=O), 1614 (C=N). ^{1}H-NMR ($CDCl_3$): δ 3.94 (s, 2H, –$\underline{CH_2}$–NH_2), 5.30 (s, 2H, Ph–CH_2), 7.01–7.82 (m, 8H, Ar-H). Analysis for: $C_{20}H_{17}N_5O_2$, Found, %: C 66.66; H 4.38; N 19.32. Calc, %: C 66.84; H 4.77; N 19.49.

N-(2-pyrolylmethylene)-1-(4-isopropylbenzyl)-benzimidazol-2-methanamine (7c). Brownish solid; 67% yield; mp 169–171°C; IR (KBr): 1643 (C=O), 1586 (C=N). ^{1}H-NMR ($CDCl_3$): δ 1.08 (d, 6H, –$CH(CH_3)_2$), 3.83 (s, 3H CH_3), 5.58 (s, 2H, Ph–CH_2), 6.98–7.20 (m, 8H, Ar-H). Analysis for: $C_{23}H_{24}N_4$. Found, %: C 77.96; H 6.89; N 15.22. Calc, %: C 77.50; H 6.79; N 15.72.

Preparation of compound (8). To a suspension of (**2b**, 5 mmol) and 10% Pd-C (0.25 g) in methanol (2–5 mL), 90% formic acid (2.5 mL) was added. The resulting solution was stirred at room temperature for 4 h, and the mixture was filtered through celite and washed with methanol. The filtrate evaporated under reduced pressure, suspended in water, and neutralized with ammonia. The resulting solid was extracted with ether and dried over anhydrous Na_2SO_4. The organic layer on evaporation afforded **8** (76%) as yellow solid, IR (KBr): 1450, 1360, 720. ^{1}H-NMR ($CDCl_3$): δ 5.44 (s, 2H, CH_2), 7.10–7.49 (m, 8H, Ar-H).

N-(2-pyrolylmethylene)-4-(2-chlorobenzimidazolyl)methyl benznamine (9). A mixture of pyrrole-2-carboxaldehyde (10 mmol) and **8** (10 mmol) in methanol (10 ml) containing three drops of glacial acetic acid was heated to reflux for 1.5 h. The Schiff base (**9**) was precipitated on cooling at 5°C. The solid was filtered off and recrystallized from EtOAc to give white solid (56%) yield; mp 145–147 °C; ^{1}H-NMR ($CDCl_3$, δ, ppm): 5.08 (s, 2H, Ph–CH_2), 6.81–7.57 (m, 3H, CH-pyrrole), 7.12–7.28 (m, 8H, Ar-H). Analysis for: $C_{19}H_{15}ClN_4$. Found, %: C 68.66; H 4.34; N 16.87. Calc, %: C 68.16; H 4.52; N 16.73.

Synthesis of 4-(2-pyrrolyl methylene amino)-1,2-dihydro-1, 5-dimethyl-2-phenylpyrazol-3-one (11). 2-Pyrrole carboxaldehyde (10 mmol) and few drops of glacial acetic acid were added to a solution of 4-amino-1,5-dimethyl-2-phenylpyrazol-3-one (**10**) (10 mmol) in anhydrous ethanol (20 mL) at room temperature. The reaction mixture was refluxed for 2 h and then cooled at room temperature. The solid formed was filtered and washed with ether to provide the desired Schiff base (**11**): yellow solid (82.7%), mp 194–195°C; IR (KBr, cm^{-1}):1665 (C=O), 1615 (C=N). ^{1}H-NMR ($CDCl_3$): δ 2.41 (s, 3H, =C–CH_3), 3.09 (s, 3H, N–CH_3), 7.05–7.15 (m, 3H, CH-pyrrole), 7.26–7.49 (m, 5H, Ar-H), 10.75 (s, 1H, CH=N), 11.35 (s, 1H, NH).

Synthesis of N-substituted-benzyl-pyrroleaminopyrine Schiff bases (12a–c). NaH (60% in mineral oil, 11.8 mmol) was added to a solution of **11** (10 mmol) in DMF at 0°C. The mixture was stirred at 0°C for 30 min, and 4-substituted benzyl chloride (10 mmol) was added. The mixture was stirred at room temperature for 8 h, acidified with saturated aqueous solution of NH_4Cl, and extracted twice with EtOAc. The organic layers were combined, washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography.

4-((1-(4-Fluorobenzyl)-1H-pyrrol-2-yl)methylene-amino)-1,2-dihydro-1,5-dimethyl-2-phenyl-pyrazol-3-one (12a). Yellow solid (89%), mp 165–168°C; IR (KBr): 1655 (C=O), 1605 (C=N). ^{1}H-NMR ($CDCl_3$): δ 2.47 (s, 3H, =C–CH_3), 3.18 (s, 3H, N–CH_3), 5.58 (s, 2H, Ph–CH_2), 7.05–7.15 (m, 3H, CH-pyrrole),6.97–7.82 (m, 9H, Ar-H), 10.77 (s, 1H, CH=N). ^{13}C-NMR ($CDCl_3$, δ, ppm): 124.19, 129.08, 126.70, 149.20,131.17, 134.85, 160.88 (C=O), 156.76 (CH=N), 131.71 ($C_{(py)}$), 110.41 ($C_{(py)}$), 108.08 ($C_{(py)}$), 122.57 ($C_{(py)}$), 134.8, 130.5, 128.6, 131.2, 10.09 (C–$\underline{CH_3}$), 35.85 (CH_3–N), 50.42 (Ph–$\underline{CH_2}$–N). Analysis for: $C_{23}H_{21}FN_4O$, Found, %: C 71.52; H 5.18; N 14.80. Calculated, %: C 71.12; H 5.45; N 14.42.

4-((1-(4-Nitrobenzyl)-1H-pyrrol-2-yl)-methylene-amino)-1, 2-dihydro-1,5-dimethyl-2-phenyl-pyrazol-3-one (12b). Orange solid (68%), mp 155–158°C; IR (KBr):1660 (C=O), 1614 (C=N). ^{1}H-NMR ($CDCl_3$): δ 2.45 (s, 3H, =C–CH_3), 3.18 (s, 3H, N–CH_3), 5.85 (s, 2H, Ph–CH_2), 6.65–7.35 (m, 3H, CH-pyrrole),7.30–7.90 (m, 9H, Ar-H), 10.85 (s, 1H, –CH=N). ^{13}C-NMR ($CDCl_3$, δ, ppm): 124.19, 129.08, 126.70, 149.20,131.17, 134.85, 160.88 (C=O), 156.76 (CH=N), 131.71 ($C_{(py)}$), 110.41 ($C_{(py)}$), 108.08 ($C_{(py)}$), 122.57 ($C_{(py)}$), 142.78, 130.0, 121.6, 145.2, 10.09 (C–$\underline{CH_3}$), 35.85 (CH_3–N), 50.42 (Ph–$\underline{CH_2}$–N). Analysis for: $C_{23}H_{21}N_5O_3$, Found, %: C 66.43; H 5.12; N 16.64. Calc, %: C 66.49; H 5.09; N 16.86.

4-((1-(4-Isopropylbenzyl)-1H-pyrrol-2-yl)methylene-amino)-1,2-dihydro-1,5-dimethyl-2-phenyl-pyrazol-3-one (12c). Yellow solid (89%), mp 165–168°C; IR (KBr): 1655 (C=O), 1605 (C=N). ^{1}H-NMR ($CDCl_3$): δ 2.47 (s, 3H, =C–CH_3), 3.18 (s, 3H, N–CH_3), 5.58 (s, 2H, Ph–CH_2), 7.05–7.15 (m, 3H, CH-pyrrole),6.97–7.82 (m, 9H, Ar-H), 10.77 (s, 1H, CH=N). ^{13}C-NMR ($CDCl_3$, δ, ppm): 124.19, 129.08, 126.70, 149.20,131.17, 134.85, 160.88 (C=O), 156.76 (CH=N), 131.71 ($C_{(py)}$), 110.41 ($C_{(py)}$), 108.08 ($C_{(py)}$), 122.57 ($C_{(py)}$), 134.8, 130.5, 128.6, 131.2, 10.09 (C–$\underline{CH_3}$), 35.85 (CH_3–N), 50.42 (Ph–$\underline{CH_2}$–N). Analysis for: $C_{26}H_{28}N_4O$. Found, %: C 75.62; H 6.16; N 13.87. Calc, %: C 75.70; H 6.84; N 13.58.

Synthesis of 2-pyrrolylmethylene-5-phenyl-1,3,4-oxadiazol-2-amine (14). 2-Pyrrole carboxaldehyde (10 mmol) and few drops of glacial acetic acid were added to a solution of **13** (10 mmol) in anhydrous ethanol (20 mL) at room temperature. The reaction mixture was refluxed for 2 h and then cooled at room temperature. The solid formed was filtered and washed with ether to provide the desired Schiff base. **(14)**: white solid, yield (74%), mp 203–205° C; ^{1}H NMR (DMSO): δ 7.68–7.45 (m, 5H, Ar-H), 8.73 (s, 1H, CH=N) ppm. IR (KBr): 1713, 1618, 1582, 1391, 1293, 1245, 1023, 694 cm^{-1}.

Synthesis of N-substituted benzyl pyrrole-1,3,4-oxadiazole Schiff bases (15a–c): General procedure. To an ice-cooled solution of **14** (10 mmol) in DMF was added NaH (60% in mineral oil, 11.8 mmol), and the mixture was stirred at 0°C for 30 min. To the mixture were added 4-substituted benzyl chlorides (10 mmol) at 0°C and stirred at room temperature for 8 h. The mixture was acidified with saturated aqueous solution of NH_4Cl and extracted twice with EtOAc. The organic layers were combined, washed with brine, dried over anhydrous $MgSO_4$, and concentrated under vacuum. The residue was purified by silica gel column chromatography.

N-((1-(4-Fluorobenzyl)-1H-pyrrol-2-yl)methylene)-5-phenyl-1,3,4-oxadiazol-2-amine (15a). Yellow solid (89%), mp 165–168°C; ^{1}H-NMR ($CDCl_3$): δ 5.58 (s, 2H, Ph–CH_2), 7.05–7.15 (m, 3H, CH-pyrrole), 6.97–7.82 (m, 9H, Ar-H), 10.77 (s, 1H, CH=N). Analysis for: $C_{20}H_{15}FN_4O$, Found, %: C 69.36; H 4.08; N 16.52. Calc, %: C 69.35; H 4.37; N 16.18.

N-((1-(4-nitrobenzyl)-1H-pyrrol-2-yl)methylene)-5-phenyl-1,3,4-oxadiazol-2-amine (15b). Orange solid (68%), mp 155–158°C; ^{1}H-NMR ($CDCl_3$): δ 5.85 (s, 2H, Ph–CH_2), 6.65–7.35 (m, 3H, CH-pyr), 7.30–7.90 (m, 9H, Ar-H), 10.85 (s, 1H, –CH=N). Analysis for: $C_{20}H_{15}N_5O_3$, Found, %: C 64.12; H 4.36; N 18.43. Calc, %: C 64.34; H 4.05; N 18.76.

N-((1-(4-isopropylbenzyl)-1H-pyrrol-2-yl)methylene)-5-phenyl-1,3,4-oxadiazol-2-amine (15c). Yellow crystal (91%); mp 168–169°C; ^{1}H-NMR ($CDCl_3$): δ 3.83 (s, 3H CH_3), 6.81–7.57 (m, 9H, Ar-H), 10.41 (s, 1H, –N=CH). Analysis for: $C_{23}H_{22}N_4O$, Found, %: C 74.38; H 5.41; N 15.06. Calc, %: C 74.57; H 5.99; N 15.12.

Acknowledgments. The authors are grateful to the National Institute of Health, the National Institute of General Medical Sciences, the MBRS Program (GM 08111), and the Research Center at Minority Institutions Grant (RCMI) RR 03020. Also, much gratitude goes to the FAMU School of Graduate Studies, Faculty Research Award Program.

REFERENCES AND NOTES

[1] Kumar, V.; Kaue, K.; Gupta, G. K.; Sharma, A. K. Eur J Med Chem 2013, 69, 735.
[2] Spasov, A.; Yozhitsa, I.; Bugaeva, L. I.; Amisimova, V. A. Pharm Chem J 1999, 33, 232.
[3] Gaba, M.; Singh, S.; Mohan, C. Eur J Med Chem 2014, 76, 494.
[4] Khokra, S. I.; Choudhary, D. Asian J Biochem Pharm Res 2011, 3, 476.
[5] Kharb, R.; Shahar Yar, M.; Sharma, P. C. Mini Rev Med Chem 2011, 11, 84.
[6] Keri, R. S.; Patil, M. R.; Patil, S. A.; Budagumpi, S. Eur J Med Chem 2015, 89, 207.
[7] Khalilullah, H.; Ahsan, M. J.; Hedaitullah, M.; Khan, S.; Ahmed, B. Mini Rev Med Chem 2012, 12, 789.
[8] Zarighi, A.; Hajimahdi, Z. Expert Opin Ther Pat 2013, 23, 1209.
[9] Kharb, R.; Yar, M. S.; Sharma, P. C. Curr Med Chem 2011, 18, 3265.
[10] Bansal, Y.; Silakari, O. Bioorg Med Chem 2012, 20, 6208.
[11] Ansari, K. F.; Lal, C. Eur J Med Chem 2009, 44, 4028.
[12] Krishnanjaneyulu, I. S.; Saravanan, G.; Vamsi, J.; Supriya, P.; Bhavana, J. U.; Sunil Kumar, M. V. J Adv Pharm Technol Res 2014, 5, 21.
[13] Pace, A.; Pierro, P. Org Biomol Chem 2009, 7, 4337.
[14] De Oliveira, C. S.; Lira, B. F.; Barbosa-Filho, J. M.; Lorenzo, J. G.; de Athayde-Filho, P. F. Molecules 2012, 17, 10192.
[15] Sun, J.; Makawana, J. A.; Zhu, H. L. Mini Rev Med Chem 2013, 13, 1725.
[16] Basoglu, S.; Yolal, M.; Demirci, S.; Demirbas, N.; Bektas, H.; Karaoglu, S. A. Acta Pol Pharm 2013, 70, 229.
[17] Chawla, R.; Arora, A.; Parameswaran, M. K.; Chan, P.; Sharma, D.; Michael, S.; Ravi, T. K. Acta Pol Pharm 2010, 67, 247.
[18] Saha, R.; Tanwar, O.; Mareella, A.; Alam, M. M.; Akhter, M. Mini Rev Med Chem 2013, 13, 1027.
[19] Bondock, S.; Rabie, R.; Etman, H. A.; Fadda, A. Eur J Med Chem 2008, 43, 2122.
[20] Marella, A.; Ali, M. R.; Alam, M. T.; Saha, R.; Tanwar, O.; Akhter, M.; Shaquiquzzaman, M.; Alam, M. M. Mini Rev Med Chem 2013, 13, 921.
[21] Summit, S.; Balasubramanian, N.; Vasudevan, M.; Rakesh, K. M.; Abu Baker, A. M. Med Chem Res 2012, 21, 3863.
[22] Ghorab, M. M.; El-Gazzar, M. G.; Alsaid, M. S. Int J Mol Sci 2014, 15, 7539.
[23] Kumar, V.; Kaur, K.; Gupta, G. K.; Shama, A. K. Eur J Med Chem 2013, 69, 735.
[24] Khalil, N. A.; Ahmed, E. M.; Mohamed, K. O.; Nissan, Y. M.; Zaitone, S. A. Bioorg Med Chem 2014, 22, 2080.
[25] Pedro, M.; Santos, M. P.; Alexandra, M.; Antunes, M.; Noronha, J.; Fernandes, E.; Abel, J.; Veira, S. C. Eur J Med Chem 2010, 45, 2258.
[26] Metwally, M. A.; Gouda, M. A.; Ammar, N.; Harmal, A.; Khalil, M. Eur J Med Chem 2012, 56, 254.
[27] Gopishetty, B.; Zhang, S.; Kharkar, P. S.; Antonio, T.; Reith, M.; Dutta, A. K. Bioorg Med Chem 2013, 21, 3164.
[28] Dutta, A. K.; Venkataraman, S. K.; Fei, X. S.; Kolhatkar, R.; Zhang, S.; Reith, M. E. Bioorg Med Chem 2004, 12, 4361.
[29] Vijesh, A. M.; Isloor, A. M.; Shetty, P.; Sundershan, S.; Fun, H. K. Eur J Med Chem 2013, 62, 410.
[30] Xu, Y.-L.; Lin, H.-Y.; Cao, R.-J.; Ming, Z.-Z.; Yang, W.-C.; Yang, G.-F. Bioorg Med Chem 2014, 22, 5194.
[31] Wang, X.-Q.; Liu, L. X.; Li, Y.; Sun, C. J.; Chen, W.; Li, L.; Zhang, H. B.; Yang, X. D. Eur J Med Chem 2013, 62, 111.