Synthesis of Certain 2-Substituted-1*H*-benzimidazole Derivatives as Antimicrobial and Cytotoxic Agents

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A series of 2-substituted-1*H*-benzimidazole derivatives were synthesized and evaluated for antimicrobial, antifungal and cytotoxic activities. The results showed that all tested compounds showed potent antimicrobial activity against some species of Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*) and fungi (*Candida albicans*) with minimum inhibitory concentrations (MICs) lower than 0.016 μg/mL. In contrast, all tested compounds were inactive against *Staphylococcus aureus* (Gram-positive bacterium). The final targets were also tested for their antitumor activity *in vitro* on cervical carcinoma (HeLa) cell line. Eight of the test compounds displayed more potent cytotoxic effect than doxorubicin at nanomolar concentrations. Compounds 2c and 3c exerted the strongest cytoyoxic effect with IC_{50} 15 and 13 nM, respectively.

Key words benzimidazole; synthesis; antimicrobial; cytotoxic

Benzimidazole ring is an important pharmacophore in drug discovery. Extensive biochemical and pharmacological studies have confirmed that benzimidazole molecule is associated with a wide range of biological activities including anticancer,[1,2] antiviral,[3,4] antibacterial,[5–7] antifungal,[8,9] antihelmintic,[10] antioxidant,[11,12] antihypertensive,[13] and anticoagulant,[14] properties.

During the past decades, the human population had been affected with life-threatening infectious diseases caused by multidrug-resistant Gram-positive and Gram-negative pathogen bacteria.[15] Moreover, the long term use of several drugs to treat microbial infections may cause serious health problems, especially in patients with impaired liver or kidney functions.[16] Therefore, there is an increasing need to design new antibacterial and antifungal agents with better activity and higher safety profile. Due to the structural similarity to purine, antibacterial ability of benzimidazoles is explained by their competition with purines resulting in inhibition of the synthesis of bacterial nucleic acids and proteins.[17]

Literature survey has shown that among the benzimidazole derivatives, the 2-substituted ones are pharmacologically more potent as antibacterial and antifungal agents and hence, the design and synthesis of 2-substituted benzimidazoles are the potential area of research.[18]

Besides, both Schiff bases and azo compounds are important pharmacophores in the medicinal and pharmaceutical fields. Certain compounds incorporating an azo moiety in their structures have shown antibacterial, antifungal, and antitumor activities.[19,20] Also, it has been suggested that the azomethine linkage might be responsible for the biological activities displayed by Schiff bases.[21–23] Recently, certain 2-substituted benzimidazole Schiff bases were found to display potent anti-proliferative activity against HeLa and MCF-7 cell lines.[3]

From the view point of molecular design, the combination of two biologically active molecules or pharmacophores is a well-known approach for the build-up of drug-like molecules,[24,25] which allows us to find more potent agents. In light of the antimicrobial, antifungal and antiproliferative importance of benzimidazole, azo compounds and Schiff bases it was thought that it would be of interest to synthesize a single molecule containing more than one pharmacophore (hybrids or conjugates). These merged pharmacophores, may be addressing the active site of different targets for the purpose to overcome drug resistance, as well as reducing unwanted side effects.[24]

The present work comprises the combination of 2-aminobenzimidazole pharmacophore with various substituted aromatic or heterocyclic rings *via* azo or azomethine linker, (compounds **2a–d**, **3a–c**, **4**, **5a**, **b**) (Charts 1–3), to study the potential additive effect of the combined molecule towards antimicrobial and cytotoxic activities. It was interesting to synthesize hybrids between 2-aminobenzimidazole pharmacophore and certain substituted pyrazoles possessing anticancer and antimicrobial activities[26–28] or the common analgesic drug, floctafenine to give compounds **3a–c**, respectively (Chart 2), hoping that this combination may possess a potential analgesic activity along with possible anticancer effect. In light of anticancer activity observed by indole-barbituric acid hybrids,[25,29] benzimidazole-barbituric acid and benzimidazole-thiobarbituric acid hybrids were synthesized, (compounds **5a**, **b**) Furthermore, cyclization of the 2-aminobenzimidazole to the corresponding imidazotriazine ring system, (compounds **8**, **9**) (Chart 4) may enhance the potential anticancer profile.[30,31]

Results and Discussion

Chemistry The reaction sequence employed for the preparation of target compounds **2a–d** and **3a–c** is shown in Chart 1.

Title compounds **2a–d** were prepared in 55–70% yields by coupling of the diazotized 2-amino-1*H*-benzimidazole (**1**), commercially available, with various phenolic or an active methylene compounds in basic medium following reported procedure.[32] IR spectra of compounds **2a–d** showed

The authors declare no conflict of interest.

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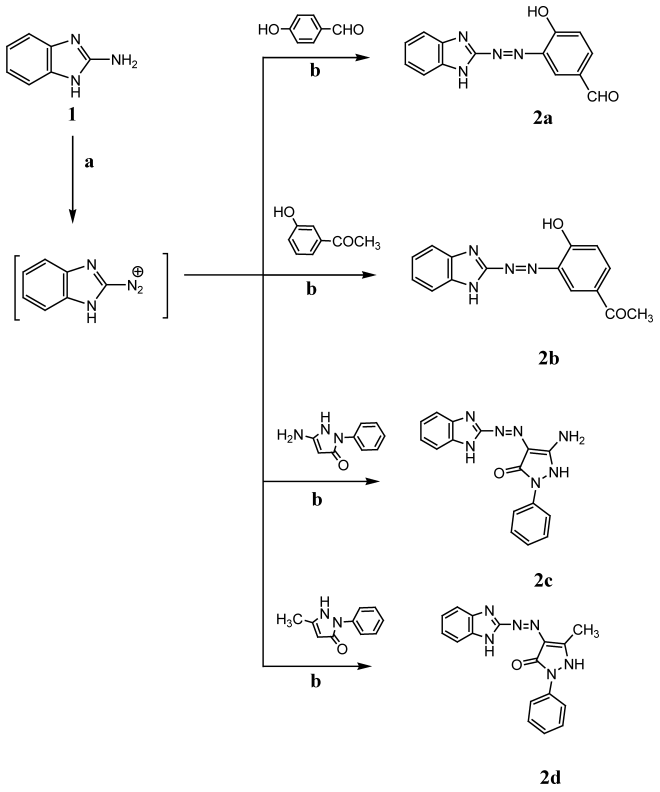
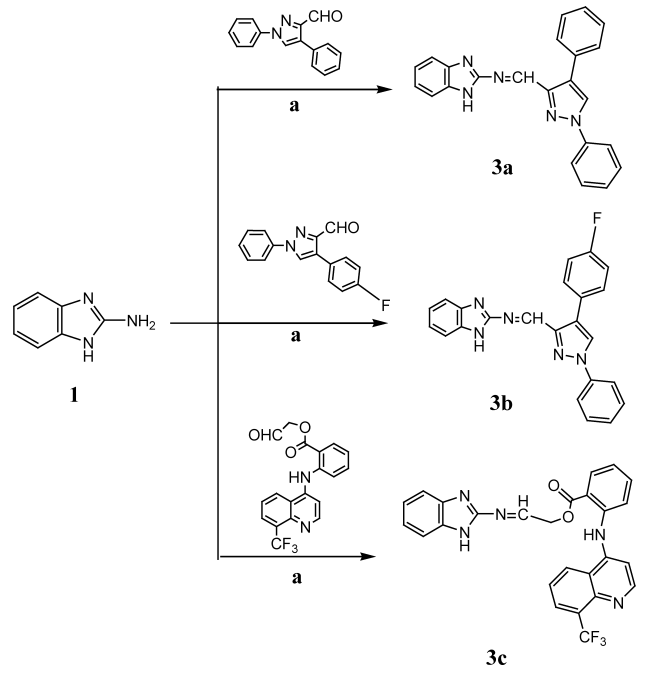


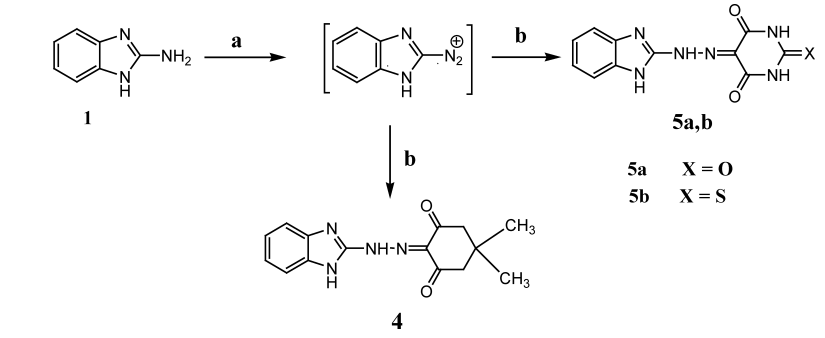
Reagents and conditions: (a) $NaNO_2$, HCl, −5°C, (b) −5°C.

Chart 1



Reagents and conditions: (a) Abs. ethanol, gl. Acetic acid, 13 h.

Chart 2



Reagents and conditions: (a) $NaNO_2$, HCl, −5°C, (b) dimedone, barbituric or thiobarbituric acids, NaOH, −5°C.

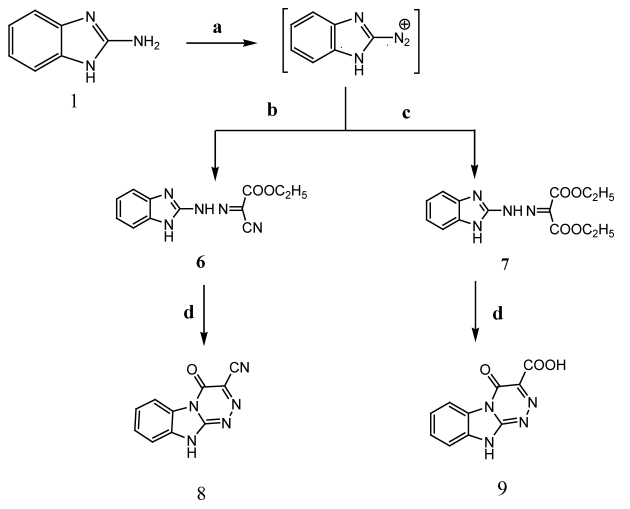
Chart 3

disappearance of absorption bands for (NH_2) and appearance of bands in the range of 1681–1732 cm^{-1} which confirmed the presence of carbonyl function. Furthermore, ^{1}H-NMR spectra of **2b** and **2d** showed a singlet signal for CH_3 protons at 2.45 and 2.31 ppm respectively. In the ^{1}H-NMR spectra of compounds **2a** and **2b**, additional OH signals (D_2O exchangeable) were observed at 10.05 and 10.58 ppm, however, the signal belonging to NH of pyrazolone ring did not appear in either of compounds **2c** or **2d**. Other ^{1}H-NMR signals and mass spectra were consistent with the proposed structures.

On the other hand, the target benzimidazole Schiff bases **3a**–**c** were prepared in 75–80% yields by heating 2-amino-1*H*-benzimidazole (**1**) and the corresponding aromatic aldehydes in acid medium for 13 h at reflux. The structures of all synthesized Schiff bases were determined by spectral and microanalytical analyses. IR spectrum of **3c** displayed a band at 1685 cm^{-1} due to carbonyl function. The ^{1}H-NMR spectra of **3a** and **3b** have shown new singlet signals at δ 9.33 and 9.28 ppm respectively corresponding to the pyrazole proton. All the other aromatic and aliphatic protons were observed in the expected regions. The title compounds were further confirmed by mass spectral data which showed the molecular ion peak and explained some possible fragmentation pattern of these compounds.

Moreover, compounds **4**, **5a** and **5b** were prepared in 60–75% yields by coupling of the diazotized **1** with the active methylene compounds namely, dimedone, barbituric and thiobarbituric acids respectively. IR spectra exhibited very similar features and showed the expected bands for the characteristic groups which are present in the compounds such as NH stretching vibrations, amide C=O stretching, and another specific band for C=S vibrations in **5b**.

Chart 3 deals with the preparation of the target benzimidazotriazine derivatives **8** and **9**. Coupling of the diazotized **1**



Reagents and conditions: (a) $NaNO_2$, HCl, −5°C, (b) $CNCH_2COOC_2H_5$, CH_3COONa, −5°C, (c) $H_2C(COOC_2H_5)_2$, CH_3COONa, −5°C, (d) 65% CH_3COOH, 30 min.

Chart 4

with ethyl cyanoacetate or diethylmalonate afforded the hydrazono derivatives **6** and **7** in 70% and 65% yields respectively. Their IR spectra revealed C=O band near 1700 cm^{-1}. Additionally, compound **6** displayed a band at 2218 cm^{-1} due to CN function. ^{1}H-NMR spectra were consistent with the proposed structures. Two different signals of imidazole NH and hydrazone NH were observed around δ 6.5 and 10.5 ppm respectively. Cyclization of the latter compounds by heating in 65% acetic acid afforded the final targets **8** and **9** in 60% and 63% yields respectively. Additionally, heating of **7** with 65% acetic acid resulted in hydrolysis of the ester function, as substantiated by IR spectroscopy, which revealed two bands of C=O at 1701 and 1680 cm^{-1}. NH and OH of the acid **9** appeared as a broad band at 3417–2681 cm^{-1}. ^{1}H-NMR analysis displayed a D_2O exchangeable signal at δ 12.43 ppm corresponding to acid OH, which confirms the hydrolysis of the ester function. All other aliphatic and aromatic protons are observed in the expected regions.

Antimicrobial Evaluation All the synthesized compounds **2a–d**, **3a–c**, **4**, **5a**, **b**, **6**, **7**, **8**, **9** were screened for their *in vitro* antimicrobial activity against Gram-negative bacteria including *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, Gram-positive bacterium (*Staphylococcus aureus*) and fungi (*Candida albicans*) by agar dilution method.[33] Tobramycin and voriconazole were used as a reference drugs.

The evaluation of test compounds for antibacterial activity showed promising results, specifically against Gram-negative organisms and *Candida albicans*, with very low minimum inhibitory concentrations (MICs) (<0.016 μg/mL). The observed MIC values were lower than those recorded by reference drugs. In contrast, Gram-positive bacterium (*Staphylococcus aureus*) was found to be resistant to all test compounds with MIC >256 μg/mL compared to tobramycin as a positive control (1 μg/mL). Furthermore, our data demonstrated that *Candida albicans* was sensitive to all tested compounds, where the recorded MICs were <0.016 μg/mL, which was lower than the breakpoint of voriconazole (0.12 μg/mL).

In summary, all tested compounds showed antimicrobial activity against Gram-negative bacteria and *Candida albicans*, however, they were inactive against *Staphylococcus aureus*, Table 1.

***In-Vitro* Antitumor Evaluation** Compounds **2a–d**, **3a–c**, **4**, **5a**, **b**, **8** and **9** were screened for their antiproliferative activity on cervical carcinoma (HeLa) cell line using Sulforhodamine B (SRB) colorimetric assay, in comparison with doxorubicin as a reference drug.

The cytotoxic activities are expressed by median growth inhibitory concentration (IC_{50}) and provided in Table 2. The results are represented graphically in Figs. 1–4. From the results, it is evident that most tested compounds exerted potent to moderate growth inhibitory activity in nanomolar concentration, in particular compounds **2c** and **3c** (IC_{50}=15, 13 nM respectively). Eight of the test compounds displayed more potent cytotoxic activity compared to doxorubicin with IC_{50} range 13–37 nM, Table 2, Figs. 1–4.

Conclusion

Although the number of tested compounds in this study is limited, some structural features that are important for explanation of their cytotoxic effects can be referred.

Within the azo derivatives **2a–d,** the 3-aminopyrazolone

Table 1. MICs (μg/mL) of the Test Compounds against Various Clinical Isolates

Compound	Pseud.	E.c.	Sal.	St.	Ca.
2a	<0.016	<0.016	<0.016	>256	<0.016
2b	<0.016	<0.016	<0.016	>256	<0.016
2c	<0.016	<0.016	<0.016	>256	<0.016
2d	<0.016	<0.016	<0.016	>256	<0.016
3a	<0.016	<0.016	<0.016	>256	<0.016
3b	<0.016	<0.016	<0.016	>256	<0.016
3c	<0.016	<0.016	<0.016	>256	<0.016
3d	<0.016	<0.016	<0.016	>256	<0.016
4	<0.016	<0.016	<0.016	>256	<0.016
5a	<0.016	<0.016	<0.016	>256	<0.016
5b	<0.016	<0.016	<0.016	>256	<0.016
8	<0.016	<0.016	<0.016	>256	<0.016
9	<0.016	<0.016	<0.016	>256	<0.016
Tobramycin	1	1.29		1	—
Voriconazole	—	—	—	—	0.12

Pseud: *Pseudomonas aeruginosa*, E.c.: *Escherichia coli*, Sal.: *Salmonella typhi*, St.: *Staphylococcus aureus*, Ca.: *Candida albicans*.

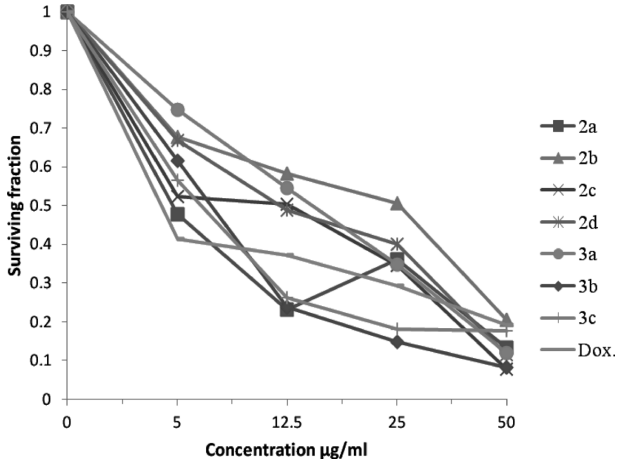


Fig. 1. Cytotoxicity of **2a**–**d**, **3a**–**c** and Doxorubicin against Cervical Carcinoma Cell Line (HeLa)

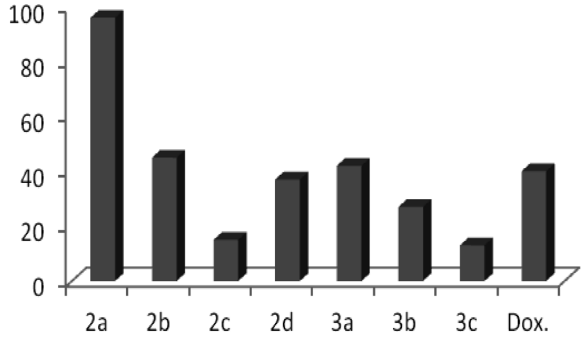


Fig. 2. IC_{50} Values of **2a**–**d** and **3a**–**c**, and Doxorubicin against HeLa

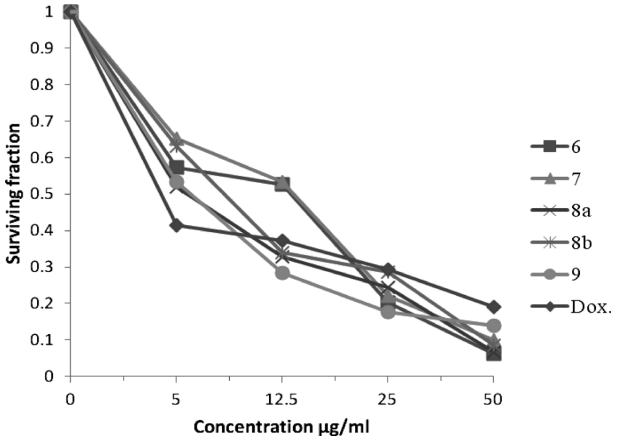


Fig. 3. Cytotoxicity of **6**, **7**, **8a**, **b**, **9**, and Doxorubicin against Cervical Carcinoma Cell Line (HeLa)

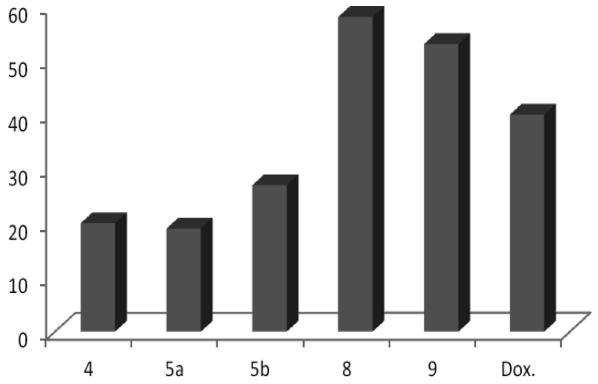


Fig. 4. IC_{50} Values of **6**, **7**, **8a**, **b**, **9** and Doxorubicin against HeLa

derivative **2c** was found to show superior anticancer activity against HeLa cell line in comparison to its 3-methyl analogue, **2d**. This could be assigned to the presence of amino function, which favours the potency over the methyl group in compound **2d**.[34] Additionally, the pyrazole ring in compounds **2c** and **2d** plays an important role in enhancing the anticancer activity.[26–28] On the other hand, the phenolic compounds **2a** and **2b** displayed lower activity.

Furthermore, within the Schiff bases **3a**–**c**, it was envisioned that compound **3c** showed most potent cytotoxicity with IC_{50}=13 nM. This may be attributed to the presence of aminoquinoline moiety, which was reported to possess anticancer activity.[35] Moreover, the 4-fluoro derivative **3b** exhibited appreciable cytotoxic activity with IC_{50}=27 nM. compared to the unsubstituted analogue **3a**. This result may be substantiated on the basis of previous publications which demonstrated the high anti-tumor activities of fluorine-containing compounds. The high electronegativity of fluorine atom attached to aromatic ring manipulate the magnitude of activity.[36,37] Besides, the hydrazono derivatives **4** and **5a**, **b** showed more potent cytotoxic activity than doxorubicin with IC_{50} range 19–27 nM. The cyclohexanedione derivative **4** and the pyrimidinetrione derivative **5a** were almost equipotent, however, incorporation of carbonyl group in the position 2 of pyrimidine scaffold by thioxo function, compound **5b**, resulted in decline in cytotoxic activity.[38] On the other hand, the benzimidazotriazine derivatives **8** and **9** showed moderate potency compared to doxorubicin.

Table 2. *In Vitro* Cytotoxic Activity of Some of the Synthesized Compounds against Human Breast Cancer Cell Line HeLa

Compound No.	HeLa (IC_{50})[a,b] nM
2a	96
2b	45
2c	15
2d	37
3a	42
3b	27
3c	13
4	20
5a	19
5b	27
8	58
9	53
Doxorubicin	40

a) IC_{50}: dose of the compound which inhibit tumor cell proliferation by 50%. *b*) Values are means of three experiments.

Experimental

Chemistry Melting points were determined on Griffin apparatus and the values given are uncorrected. IR spectra were determined on Shimadzu IR 435 spectrophotometer (KBr, cm^{-1}). ^{1}H-NMR spectra were carried out using a Varian Mercury-300 (300 MHz) Spectrophotometer using TMS as internal standard. Chemical shift values are recorded in ppm on δ scale, Microanalytical Center, Cairo University, Egypt.

Mass spectra were recorded on a GCMP-QP1000 EX Mass spectrometer, Microanalytical Center, Cairo University, Egypt. Elemental analyses were carried out at the Microanalytical Center, Cairo University, Egypt. Progress of the reactions was monitored using TLC sheets precoated with UV fluorescent silica gel Merck 60F 254 using acetone–benzene (1 : 9) and were visualized using UV lamp.

The starting materials 3-amino-1-phenyl-1,2-dihydropyrazol-5-one and 3-methyl-1-phenyl-1,2-dihydropyrazol-5-one are commertially available. 1,4-Diphenyl-1*H*-pyrazole-3-carbaldehyde, 4-(4-fluoroiphenyl)-1-phenyl-1*H*-pyrazole-3-carbaldehyde[39] and ethyl 2-(8-trifluoromethyl)quinolin-4-ylamino)-benzoate[40] were prepared as reported.

All chemicals were obtained from Aldrich, Fluka, or Merck chemicals.

General Procedure for Compounds 2a–d, 4 and 5a,b An ice-cold solution of aryldiazonium salt [prepared from 2-aminobenzimidazole (**1**) (1.32 g, 0.01 mol), concentrated hydrochloric acid (3 mL) and sodium nitrite (0.69 g, 0.01 mol) in water (15 mL)] was added to a chilled solution of an appropriate phenolic or an active methylene compound (0.01 mol) and sodium hydroxide (1.6 g, 0.04 mol) in water (25 mL). The reaction mixture was maintained at −5°C with continuous stirring for 30 min, then acidified with glacial acetic acid till pH 5–5.5. The resulting solid was filtered, washed with water, dried and crystallized from methanol.

3-[(1*H*-Benzo[*d*]imidazol-2-yl)diazenyl]-4-hydroxybenzaldehyde (**2a**): Yield 65%; mp 297–298°C; IR (KBr) cm^{-1}: 3352–3136 (O–H and NH), 3028 (C–H aromatic), 2727 (C–H aldehyde), 1724 (C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.54 (s, 1H, NH, D_2O exchangeable), 7.20–7.87 (m, 7H, Ar-H), 9.25 (s, 1H, CH ald.), 10.58 (s, 1H, OH); MS (electron ionization (EI)) *m/z* (% rel. int.): 265 (M-1, 0.01), 134 (100). *Anal.* Calcd for $C_{14}H_{10}N_4O_2$ (266.08): C, 63.15; H, 3.79; N, 21.04; Found: C, 63.28; H, 3.55; N, 20.89.

1-{4-[(1*H*-Benzo[*d*]imidazol-2-yl)diazenyl]-3-hydroxyphenyl}ethanone (**2b**): Yield 70%; mp 269–270°C; IR (KBr) cm^{-1}: 3370–3136 (OH and NH), 3028 (C–H aromatic), 2928, 2850 (C–H aliphatic), 1732 (C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.45 (s, 3H, CH_3), 6.80 (s, 1H, NH, D_2O exchangeable), 6.91–7.84 (m, 7H, Ar-H), 10.58 (s, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (% rel. int.): 280 ($M^{+\cdot}$, 6.95), 266 (9.00), 149 (40.33), 134.05 (72.66). *Anal.* Calcd for $C_{15}H_{12}N_4O_2$ (280.10): C, 64.28; H, 4.32; N, 19.99; Found: C, 64.16; H, 4.68; N, 20.24.

4-[(1*H*-Benzo[*d*]imidazol-2-yl)diazenyl]-3-amino-1-phenyl-1*H*-pyrazol-5(4*H*)-one (**2c**): Yield 55%; mp 253–254°C; IR (KBr) cm^{-1}: 3421, 3332, 3217 (NH_2 and NH), 3120 (C–H aromatic), 2943, 2850 (C–H aliphatic), 1681 (C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.42 (s, 2H, NH_2, D_2O exchangeable), 6.62 (s, 1H, NH, D_2O exchangeable), 6.90–6.94 (m, 1H, Ar-H), 7.03–7.16 (m, 2H, Ar-H), 7.31–7.39 (m, 3H, Ar-H), 7.81–7.90 (m, 3H, Ar-H); MS (EI) *m/z* (% rel. int.): 319 ($M^{+\cdot}$, 35.7), 188 (28.6), 132 (46.4). *Anal.* Calcd for $C_{16}H_{13}N_7O$ (319.32): C, 60.18; H, 4.10; N, 30.70; Found: C, 60.30; H, 4.26; N, 31.12.

4-((1*H*-Benzo[*d*]imidazol-2-yl)diazenyl)-3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (**2d**): Yield 65%; mp 155–156°C; IR (KBr) cm^{-1}: 3271 (NH), 3062 (C–H aromatic), 2924, 2881 (C–H aliphatic), 1685 (C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.31 (s, 3H, CH_3), 5.40 (s, 1H, NH, D_2O exchangeable), 6.96–7.91 (m, 9H, Ar-H). MS (EI) *m/z* (% rel. int.): 318 ($M^{+\cdot}$, 0.25), 133 (100). *Anal.* Calcd for $C_{17}H_{14}N_6O$ (318.33): C, 64.14; H, 4.43; N, 26.40; Found: C, 64.45; H, 4.32; N, 26.13.

General Method for Preparation of Schiff Bases 3a–c Solutions of equimolar amounts of 2-aminobenzimidazole and an appropriate aromatic aldehyde (0.01 mol each) in absolute ethanol (20 mL) and glacial acetic acid (2 mL) were heated under reflux for 13 h. After cooling, the obtained product was filtered off and recrystallized from ethanol.

N-[(1,4-Diphenyl-1*H*-pyrazol-3-yl)methylene]-1*H*-benzo[*d*]-imidazol-2-amine (**3a**): Yield 80%; mp 132–133°C; IR (KBr) cm^{-1}: 3313 (NH), 3124 (C–H aromatic); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.25 (s, 1H, NH, D_2O exchangeable), 6.83–6.86 (m, 2H, Ar-H), 7.08–7.11 (m, 2H, Ar-H), 7.42–7.60 (m, 7H, 6Ar-H and =CH), 7.91–8.01 (m, 4H, Ar-H), 9.33 (s, 1H, pyrazole); MS (EI) *m/z* (% rel. int.): 363 ($M^{+\cdot}$, 0.29), 247 (100), 116 (8.21). *Anal.* Calcd for $C_{23}H_{17}N_5$ (363.41): C, 76.01; H, 4.71; N, 19.27; Found: C, 75.88; H, 4.56; N, 19.35.

N-{[4-(4-Fluorophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl]methylene}-1*H*-benzo[*d*]imidazol-2-amine (**3b**): Yield 75%; mp 159–160°C; IR (KBr) cm^{-1}: 3248 (NH), 3124 (C–H aromatic); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.00 (s, 1H, NH, D_2O exchangeable), 7.22–7.43 (m, 4H, Ar-H), 7.52–7.68 (m, 6H, 5Ar-H and =CH), 7.90–8.05 (m, 4H, Ar-H), 9.28 (s, 1H, pyrazole); MS (EI) *m/z* (% rel. int.): 381 ($M^{+\cdot}$, 0.02), 266 (49.19), 133 (68.99), 77 (100). *Anal.* Calcd for $C_{23}H_{16}FN_5$ (381.41): C, 72.43; H, 4.23; N, 18.36; Found: C, 72.15; H, 4.52; N, 18.58.

2-[(1*H*-Benzo[*d*]imidazol-2-yl)imino]ethyl–2-[(8-(Trifluoromethyl)quinolin-4-yl)amino]benzoate (**3c**): Yield 78%; mp 137–138°C; IR (KBr) cm^{-1}: 3294, 3255 (2NH), 3197 (C–H aromatic), 2916, 2835 (C–H aliphatic), 1685 (C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 4.28 (d, 2H, OCH_2), 6.18 (s, 1H, NH, D_2O exchangeable), 7.21–7.27 (m, 4H, Ar-H), 7.65–7.76 (m, 6H, 5Ar-H and =CH), 8.08–8.19 (m, 2H, Ar-H), 8.52 (d, 1H, Ar-H), 8.68 (d, 1H, Ar-H), 10.02 (s, 1H, NH, D_2O exchangeable); MS (EI) *m/z* (% rel. int.): 490 (M+H, 63.64), 489 ($M^{+\cdot}$, 21.82), 360 (74.55), 315 (69.09), 201 (73.64), 130 (69.09). *Anal.* Calcd for $C_{26}H_{18}F_3N_5O_2$ (489.45): C, 63.80; H, 3.71; N, 14.31; Found: C, 63.48; H, 3.66; N, 14.23.

2-(2-(1*H*-Benzo[*d*]imidazol-2-yl)hydrazono)-5,5-dimethylcyclohexane-1,3-dione (**4**): Yield 74%; mp 178–179°C; IR (KBr) cm^{-1}: 3373–3365 (NH), 3059 (C–H aromatic), 2958, 2866 (C–H aliphatic), 1678, 1654 (2C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 1.21 (s, 6H, $2CH_3$), 2.43 (s, 4H, $2CH_2$), 6.02 (s, 1H, NH, D_2O exchangeable), 6.30 (s, 1H, NH, D_2O exchangeable), 6.84–7.20 (m, 4H, Ar-H); MS (EI) *m/z* (% rel. int.): 284 ($M^{+\cdot}$, 22.49), 166 (25.33), 134 (25.33). *Anal.* Calcd for $C_{15}H_{16}N_4O_2$ (284.31): C, 63.37; H, 5.67; N, 19.71; Found: C, 63.15; H, 5.55; N, 19.86.

5-(2-(1*H*-Benzo[*d*]imidazol-2-yl)hydrazono)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (**5a**): Yield 75%; mp 237–238°C; IR (KBr) cm^{-1}: 3375–3200 (NH), 3055 (C–H aromatic), 1700–1651 (3C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.04–7.30 (m, 4H, Ar-H), 7.72 (br s, 3H, 3NH, D_2O exchangeable), 10.61 (s, 1H, NH, D_2O exchangeable); MS (EI) *m/z* (% rel. int.): 273 (M+H, 0.88), 126 (4.10). *Anal.* Calcd for $C_{11}H_8N_6O_3$ (272.22): C, 48.53; H, 2.96; N, 30.87; Found: C, 48.94; H, 3.10; N, 31.12.

5-[2-(1*H*-Benzo[*d*]imidazol-2-yl)hydrazono]-2-thioxodi-

hydropyrimidine-4,6(1*H*,5*H*)-dione (**5b**): Yield 60%; mp 240–241°C; IR (KBr) cm^{-1}: 3417–3286 (NH), 3066 (C–H aromatic), 1674, 1651 (2C=O), 1215 (C=S); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.11–7.35 (m, 4H, Ar-H), 7.92 (br s, 3H, 3NH, D_2O exchangeable), 10.80 (s, 1H, NH, D_2O exchangeable); MS (EI) *m/z* (% rel. int.): 288 ($M^{+\cdot}$, 0.05), 173 (20.28), 133 (100). *Anal.* Calcd for $C_{11}H_8N_6O_2S$ (288.29): C, 45.83; H, 2.80; N, 29.15; Found: C, 46.08; H, 2.93; N, 29.31.

General Procedure for Compounds 6, 7 A mixture of an appropriate active methylene compound (0.01 mol), sodium acetate (1.08 g, 0.02 mol) in water (10 mL) was stirred for 10 min till a clear solution was formed, then chilled at −5°C. To this solution, an ice cold solution of aryldiazonium salt [prepared from 2-aminobenzimidazole (1.33 g, 0.01 mol), glacial acetic acid (7 mL) and sodium nitrite (0.69 g, 0.01 mol) in water (15 mL)] was added and the reaction mixture was maintained at −5°C for 1 h with continuous stirring. The formed precipitate was filtered, washed with water, dried then crystallized from ethanol.

Ethyl 2-[2-(1*H*-benzo[*d*]imidazol-2-yl)hydrazono]-2-cyanoacetate (**6**): Yield 70%; mp 172–173°C; IR (KBr) cm^{-1}: 3400–3252 (NH), 3082 (C–H aromatic), 2927, 2877 (C–H aliphatic), 2218 (CN), 1685 (C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 1.28 (t, 3H, CH_3), 4.09 (q, 2H, CH_2), 6.11 (s, 1H, NH, D_2O exchangeable), 7.08–7.23 (m, 4H, Ar-H), 10.66 (s, 1H, NH, D_2O exchangeable); MS (EI) *m/z* (% rel. int.): 257 ($M^{+\cdot}$, 8.00), 241 (38), 130 (100). *Anal.* Calcd for $C_{12}H_{11}N_5O_2$ (257.25): C, 56.03; H, 4.31; N, 27.22; Found: C, 55.95; H, 4.48; N, 27.18.

Diethyl 2-[2-(1*H*-Benzo[*d*]imidazol-2-yl)hydrazono]malonate (**7**): Yield 65%; mp 181–182°C; IR (KBr) cm^{-1}: 3417–3329 (N–H), 3066 (C–H aromatic), 2950, 2889 (C–H aliphatic), 1697 (C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 1.32 (t, 6H, $2CH_3$), 4.32 (q, 4H, $2CH_2$), 6.56 (s, 1H, NH, D_2O exchangeable), 7.20 (d, 2H, Ar-H), 7.35 (d, 2H, Ar-H), 8.37 (s, 1H, NH, D_2O exchangeable), 12.46 (s, 1H, NH, D_2O exchangeable); MS (EI) *m/z* (% rel. int.): 304 ($M^{+\cdot}$, 2.89), 232 (5.16). *Anal.* Calcd for $C_{14}H_{16}N_4O_4$ (304.30): C, 55.26; H, 5.30; N, 18.41; Found: C, 55.45; H, 5.66; N, 18.67.

General Procedure for Compounds 8 and 9 Compound **6** or **7** (0.01 mol) was heated under reflux in 65% acetic acid (25 mL) for 30 min. The reaction mixture was concentrated under reduced pressure to half its volume and cooled. The resulting precipitate was filtered, washed with water, dried and crystallized from ethanol.

4-Oxo-4,10-dihydrobenzo[4,5]imidazo[2,1-*c*][1,2,4]triazine-3-carbonitrile (**8**): Yield 60%; mp 189–190°C; IR (KBr) cm^{-1}: 3244 (NH), 3093 (C–H aromatic), 2200 (CN); 1689 (C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.51 (br s, 1H, NH, D_2O exchangeable), 6.85–6.90 (m, 2H, Ar-H), 7.08–7.13 (m, 2H, Ar-H); MS (EI) *m/z* (% rel. int.): 212 (M+H, 77.88), 211 ($M^{+\cdot}$, 9.73). *Anal.* Calcd for $C_{10}H_5N_5O$ (211.18): C, 56.87; H, 2.39; N, 33.16; Found: C, 57.15; H, 2.52; N, 32.98.

4-Oxo-4,10-dihydrobenzo[4,5]imidazo[2,1-*c*][1,2,4]triazine-3-carboxylic Acid (**9**): Yield 63%; mp 180–181°C; IR (KBr) cm^{-1}: 3417–2681 (NH and OH), 3066 (C–H aromatic), 2927. 2889 (C–H aliphatic), 1701, 1680 (2C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.18–7.24 (m, 2H, Ar-H), 7.32–7.36 (m, 2H, Ar-H), 8.41 (s, 1H, NH, D_2O exchangeable), 12.43 (s, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (% rel. int.): 230 ($M^{+\cdot}$, 3.73), 133 (100). *Anal.* Calcd for $C_{10}H_6N_4O_3$ (230.18): C, 52.18; H, 2.63; N, 24.34; Found: C, 52.43; H, 2.86; N, 24.55.

Biological Studies. Antimicrobial Evaluation Five microbial strains were used in this study after being collected from clinical and environmental samples. All newly synthesized target compounds were evaluated for their *in vitro* antibacterial activity against *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi* as a representatives of Gram-negative bacteria and *Staphylococcus aureus* as an example of Gram-positive bacterium. They were also evaluated for their *in vitro* antifungal potential against *Candida albicans*.

MICs Determination MICs were determined according to the guidelines agar dilution method described in CLSI (formerly the National Committee for Clinical Laboratory Standards).[33] A fresh volume of 10 μL of Mueller Hinton broth (MHB) (Difco) cultures, at the level of 10^5 cfu/mL, was inoculated on the surface of Mueller Hinton agar (MHA) (Difco) plates containing two-fold serial dilution of antibiotic concentrations. The plates were then incubated at 37°C for 24 h then the growth of the microorganism was recorded as positive or negative. MIC was defined as the least concentration of antimicrobial agent that showed no growth (or less than 3 cfu/mL) after incubation at 37°C for 24 h.

***In-Vitro* Cytotoxicity Assay** The cytotoxic activity was measured *in vitro* using the SRB colorimetric assay using the method of Skehan.[41] Cells were inoculated in 96-well microtiter plate (104 cells/well) for 24 h before treatment with the compound(s) to allow attachment of cell to the wall of the plate. Test compounds were dissolved in dimethyl sulfoxide (DMSO) and diluted with saline to the appropriate volume. Different concentrations of the compound under test (0.1, 2.5, 5, 10 mmol/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48 h at 37°C and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed, and stained for 30 min with 0.4% (w/v) SRB dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid, and attached stain was recovered with Tris–ethylenediaminetetraacetic acid (EDTA) buffer. Colour intensity was measured in an enzyme-linked immunosorbent assay (ELISA) reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve for breast tumor cell line after the specified time. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated. The results are given in (Table 1), and presented graphically in (Figs. 1–4).

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