STUDIES ON ANTIMICROBIAL EVALUATION OF SOME 1-((1-(1H-BENZO[d]IMIDAZOL-2-YL)ETHYLIDENE)AMINO)-6-((ARYLIDENE)AMINO)-2-OXO-4-PHENYL-1,2-DIHYDROPYRIDINE-3,5-DICARBONITRILES

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GRAPHICAL ABSTRACT

Abstract A new series of 1-((1-(1H-benzo[d]imidazol-2-yl)ethyldiene)amino)-6-((arylidene)amino)-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitriles (4a-o) have been synthesized for the development of antimicrobial agents. Newly synthesized compounds were evaluated for their in vitro antibacterial activity against Gram-positive bacteria (Pseudomonas aeruginosa, Streptococcus pyogenes), Gram-negative bacteria (Escherichia coli, Staphylococcus aureus), and antifungal activity (Candida albicans, Aspergillus niger, Aspergillus clavatus). These compounds were characterized by infrared, $^1$H NMR, $^{13}$C NMR, and mass spectra. The synthesized compounds 4b, 4e, 4h, and 4k showed potent antimicrobial activity against tested microorganisms.

Keywords Benzimidazole; biological evaluation; MIC; 2-pyridone

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INTRODUCTION

There is a growing interest in recent years in the synthesis of benzimidazole-based heterocycles because of the significant role of the benzimidazole unit. The benzimidazole scaffold is a useful structural motif for displaying chemical functionality in biologically active molecules. Moreover, benzimidazoles have been used as “privileged” scaffolds to produce substances of interest in numerous therapeutic areas, such as antibacterial,[1] antioxidant,[2] fungicidal,[3] antihypertensive,[4] antifungal,[5–7] anti-inflammatory,[8,9] anti-HIV,[10,11] anticancer,[12–15] antiparasitic,[16] antigiardia,[17] antiproliferative,[18] antiviral,[19,20] and anthelmintic.[21] The well-known example of a benzimidazole-based drug is omeprazole (Fig. 1, Formula 1), which is used to treat peptic ulcer disease, gastroesophageal reflux disease, laryngopharyngeal reflux, and Zollinger–Ellison syndrome.

2-Pyridone derivatives have also attracted considerable attention because this skeleton is present in many compounds that have been isolated from natural substances,[22,23] having various biological activities.[24] The development of efficient synthesis of 2-pyridone is an important target in current organic synthesis.[25] Some derivatives containing a 2-oxopyridine ring system have been shown to possess useful pharmacological activities, such as milrinone (Primacor), a phosphodiesterase III inhibitor; olprinone, a cardiotonic agent,[26] and camptothecin (CPT), the DNA enzyme topoisomerase I (topo I) inhibitor. Two CPT analogs, topotecan and irinotecan,[27] have been approved and are used in cancer chemotherapy today. Pyridone-based drugs are well known as chemotherapy agents. Nothapodytine B (Fig. 1, Formula 2) is an excellent antiviral drug having 2-pyridones as core moiety. In addition, the predominance of nitrile-containing drugs has changed the treatment of several diseases because of the biocompatibility of the nitrile functionality. At present, more than 30 nitrile-containing drugs are prescribed for a diverse range of medicinal conditions and more than 20 additional nitrile-containing molecules are in clinical trials. Some shining examples of nitrile-based drugs are letrozole, etravirine, riplivirine, and lersivirine.[28] In the present program, our aim is to synthesize new molecules containing multiple heterocyclic systems and to study their characterization as well as their activities. In continuation to our previous work[29–31] and because of the medicinal importance of benzimidazole and 2-pyridone as a core moiety, we report herein the synthesis of a new class of 1-((1-(1H-benzo[d]imidazol-2-yl)ethylidene)amino)-6-(arylidene)amino)-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitriles (4a–o) and try to develop potential antimicrobials. The structures of newly synthesized compounds were elucidated on the basis of infrared (IR), 1H NMR, 13C NMR, and mass spectral analysis. These compounds were

Figure 1. Benzimidazole and 2-pyridone-based drugs.
evaluated for their antimicrobial screening on various strains of bacteria and fungi.

**RESULTS AND DISCUSSION**

**Chemistry**

The synthesis of 1-((1-(1\textit{H}-benzo[\textit{d}]imidazol-2-yl)ethylidene)amino)-6-((arylidene)amino)-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitriles (4a–o) have been carried out as follows: A mixture containing \(N\)-(1-(1\textit{H}-benzo[\textit{d}]imidazol-2-yl)ethylidene)-2-cyanoacetohydrazide (0.01 mol), 2-benzylidenemalononitrile 2 (0.01 mol), and a catalytic amount of piperidine in ethanol (99\%, 30 mL) was refluxed to obtain 1-((1-(1\textit{H}-benzo[\textit{d}]imidazol-2-yl)ethylidene)amino)-6-amino-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (3). Intermediate 3 (0.01 mol) was further treated with various aromatic aldehydes (0.01 mol) in ethanol (99\%, 30 mL). The mixture was further refluxed and the solid mass was separated to obtain novel compounds of the series 4a–o (Scheme 1).

A plausible mechanistic pathway for the formation of compounds 4a–o is suggested in Scheme 2. In the first step, hydrazone (A) underwent Michael addition with Knoevenagel product (B) and produced the intermediate (C), which further underwent intramolecular nucleophilic attack on cyanide carbon followed by annulation to yield intermediate D. The intermediate D transformed to compound E by intramolecular electron transfer to nitrogen atom. In the last step, intermediate E was transformed to targeted compounds by intermolecular nucleophilic attack on carbonyl carbons of different aromatic aldehydes (Scheme 2).
Spectral Characterization

Characterization of newly synthesized compounds of the series is carried out by IR, $^1$H NMR, $^{13}$C NMR, and mass spectra and detailed discussions are given in the Experimental section. IR spectrum of compound 4a (Fig. 2; molecular formula C$_{29}$H$_{19}$N$_7$O, molecular weight 481.17 g/mol) has given stretching vibration at 3333 cm$^{-1}$, indicating the presence of N-H stretching of secondary amine. Absorption band at 3053 cm$^{-1}$ indicates the presence of C-H stretching of aromatic hydrogen.

**Scheme 2.** Plausible mechanism pathway for the synthesis of compound 4a–o.

**Spectral Characterization**

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**Figure 2.** Compound 4a.
C-H Stretching at 2853 and 2923 cm$^{-1}$ showed the presence of -CH and -CH$_3$ groups in compound 4a. The presence of -CN stretching of aromatic ring is proved by absorption bands at 2213 cm$^{-1}$. Stretching vibrations at 1638 and 1560 cm$^{-1}$ indicate the presence of C=C, C=N in the aromatic ring. The stretching vibration appeared at 1688 gave proof of >C=O present in the molecule.

$^1$H NMR spectra of final compound 4a (Fig. 2) showed that the protons attached to C-28 carbon gave chemical shift at $\delta = 1.30$ [3H, Ar-C(CH$_3$)=N] with a singlet peak. Protons of phenyl ring gave a multiplet at $\delta = 6.8$–7.8 (14H, Ar-H) and proton at position C-19 proved the presence of imine, observed as a singlet, and chemical shift at $\delta = 9.25$ showed proof of the presence of a secondary amine (1H, -CH=N-). Proton at position C-30 was observed as a singlet and chemical shift at $\delta = 12.8$ showed the presence of a secondary amine (1H, -NH).

Chemical shifts of carbons of final compound 4a (Fig. 2) that varied from $\delta = 169.4$ to 13.6 carbon nuclei under the influence of a strong electronegative environment appeared downfield; C3 showed at 169.4 while the C28 methyl group appeared at 13.6 ppm. Carbon of two phenyl rings (C12–C16 and C21–C25) showed peaks of 128.9 to 129.3 ppm. Carbon of benzimidazole ring (C31–C37) appeared in the range 114.7 to 133.8 ppm. Carbon attached to benzimidazole ring C27, C29 appeared at 151.6 and 155.7 ppm, respectively. Two nitrile groups attached to the 2-pyridone ring showed a peak at 123.2 ppm. The ketone group present in the 2-pyridone appeared at 160.1 ppm.

**Biological Evaluation**

**Antibacterial screening.** All the newly synthesized compounds (4a–o) were evaluated for their in vitro antibacterial activity against Gram-positive bacteria *Staphylococcus aureus* and *Streptococcus pyogenes* and Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* by conventional broth microdilution method using Ampicillin as a standard drug for antibacterial activity at different concentrations of 1000, 500, 200, 100, 50, 25, and 12.5 µg ml$^{-1}$ as shown in Table 1. Among the synthesized compounds (4a–o), many of them had proven their antimicrobial potency varies from moderate to excellent. Compound 4e (-2-NO$_2$-C$_6$H$_4$) had excellent activity against *E.coli* and *S. aureus*. It is noteworthy that compound 4e (-2-NO$_2$-C$_6$H$_4$) showed the greatest inhibition at MIC = 12.5 µg ml$^{-1}$, while compound 4h (-2-Cl-C$_6$H$_4$) showed inhibition at MIC = 12.5 µg ml$^{-1}$ against *P. aeruginosa* and *S. pyogenes*. These data revealed that compound 4h (-2-Cl-C$_6$H$_4$) was highly active against both organisms. Compounds 4e (-2-NO$_2$-C$_6$H$_4$) and 4h (-2-Cl-C$_6$H$_4$) showed very good activity at MIC = 50 µg ml$^{-1}$. Compound 4e (-2-NO$_2$-C$_6$H$_4$) displayed very good activity against *P. aeruginosa* while compound 4h (-2-Cl-C$_6$H$_4$) showed very good activity against *E. coli*. Moreover, compound 4g (-4-NO$_2$-C$_6$H$_4$) exhibited very good activity against *S. aureus*. Compounds 4f (-3-NO$_2$-C$_6$H$_4$) and 4i (-3-Cl-C$_6$H$_4$) displayed good activity against *E.coli* and *S. aureus* while compound 4j (-4-Cl-C$_6$H$_4$) showed good activity against *P. aeruginosa* and *S. aureus* at MIC = 100 µg ml$^{-1}$. The remaining compounds of the series possessed feeble antibacterial activity. On the other hand, the presence of similar functional groups at the *ortho* position resulted in minor increase in antibacterial activity as compared to 4e (-2-NO$_2$-C$_6$H$_4$) and 4h (-2-Cl-C$_6$H$_4$).
Antifungal screening. Minimum inhibitory concentration (MIC) values of antifungal activity were observed against *Candida albicans*, *Aspergillus niger*, and *Aspergillus clavatus* by conventional broth microdilution method. Antifungal activity showed that compound 4k (-2-CH$_3$-C$_6$H$_4$) exhibited very good activity against *A. clavatus* at 50 µg ml$^{-1}$ MIC. When we replaced hydrogen by group like (-4-OCH$_3$-C$_6$H$_4$) in compound 4n, the activity was slightly decreased against *A. niger*. The same results exhibited in compound 4l (-3-CH$_3$-C$_6$H$_4$) possessed good activity against *C. albicans* and *A. clavatus* respectively. When hydrogen was replaced by (-2-OH-C$_6$H$_4$) in compounds 4b (-2-CH$_3$-C$_6$H$_4$) and 4k (-2-CH$_3$-C$_6$H$_4$), both displayed excellent activity against *C. albicans* and *A. niger* with twofold greater MIC (12.5–25 µg ml$^{-1}$) than the reference drug. The remaining compounds of the series showed feeble antifungal activity. Thus we have discussed and compared antifungal activity based on the standard drug griseofulvin shown in Table 1.

SAR Studies

The substitution patterns of the derivatives are carefully selected to confer different electronic environments of the molecules. The electronic nature of the substituent groups leads to significant variation in antimicrobial activity. Furthermore, considering the relationship between the structure of final compounds (4a–o) and antimicrobial property, the identity of different substituents proved to be a significant parameter for influencing the activity of reported compounds. The presence of chloro

| No. | -R         | Minimum inhibitory concentrations (MIC) in µg/ml |
|-----|------------|-----------------------------------------------|
|     |            | Bacteria          | Fungi                |
|     |            | E.c. | P.a. | S.a. | S.p. | C.a. | A.n. | A.c. |
| 4a  | -H         | >1000 | 250  | 500  | 250  | >1000 | 500  | >1000 |
| 4b  | -2-OH      | 250  | 500  | 500  | 500  | 12.5 | 25   | 250  |
| 4c  | -3-OH      | 500  | >1000 | 500  | >1000 | 1000 | 250  | 250  |
| 4d  | -4-OH      | 250  | >1000 | 1000 | 500  | >1000 | 250  | 500  |
| 4e  | -2-NO$_2$  | 12.5 | 50   | 12.5 | 50   | 500  | >1000 | 500  |
| 4f  | -3-NO$_2$  | 100  | 250  | 100  | 250  | >1000 | 250  | 500  |
| 4g  | -4-NO$_2$  | 125  | 250  | 50   | 250  | >1000 | 250  | >1000 |
| 4h  | -2-Cl      | 50   | 12.5 | 250  | 12.5 | 500  | 1000 | >1000 |
| 4i  | -3-Cl      | 100  | 250  | 100  | 500  | >1000 | 500  | 1000 |
| 4j  | -4-Cl      | 250  | 100  | 100  | 500  | 500  | 250  | 500  |
| 4k  | -2-CH$_3$  | 125  | 250  | 1000 | 500  | 25   | 12.5 | 50   |
| 4l  | -3-CH$_3$  | 125  | >1000 | 500  | 500  | 100  | 250  | 100  |
| 4m  | -4-CH$_3$  | 1000 | 100  | >1000 | 500  | 500  | 500  | 250  |
| 4n  | -4-OCH$_3$ | 500  | 250  | >1000 | 500  | >1000 | 100  | >1000 |
| 4o  | -3,4,5-(OCH$_3$)$_3$ | 250 | >1000 | >1000 | 250 | >1000 | 1000 | >1000 |
| Ampicillin | 100   | 100  | 250  | 100  | 500  | 100  | 100  |
| Griseofulvin | —    | —    | —    | —    | 500  | 100  | 100  |

*E.c., Escherichia coli MTCC 443; P.a., Pseudomonas aeruginosa MTCC 1688; S.a., Staphylococcus aureus MTCC 96; S.p., Staphylococcus pyogenes MTCC 442; C.a., Candida albicans MTCC 227; A.n., Aspergillus niger MTCC 282; A.c., Aspergillus clavatus MTCC 1323.*
and nitro substituents present at ortho position on the aromatic ring has increased the antibacterial activity of compounds compared to those of electron-donating substituents. When we have changed the substitution position to para, the compound tends to lose its potency. Incorporation of electron-donating groups such as methyl, methoxy, and hydroxy diminished the antibacterial property. The presence of hydrophilic substituents on the phenyl ring provides a positive influence on antifungal activity. In agreement with these results, electron-donating groups on the ortho-substituted position showed optimum activity. It may be observed that position of substituent on the phenyl ring clearly affected the activity (e.g., same functional groups on meta and para positions are not found to be active as the standard drug). Eventually, it can be inferred from Table 1 that a compound without any substitution does not exhibit antimicrobial activity against a panel of microorganisms.

**EXPERIMENTAL**

**Materials and Methods**

The required chemicals were purchased from E. Merck. Melting points were recorded on Gallenkamp apparatus and were left uncorrected. The completion of reaction and the purity of all compounds was checked on aluminum-coated thin-layer chromatography (TLC) plates 60F245 (E. Merck) using various solvent systems as mobile phase and visualized under ultraviolet (UV) light or iodine vapor. Elemental analysis (% C, H, N) was carried out by a Perkin-Elmer 2400 CHN analyzer. Infrared (IR) spectra were also recorded on a Perkin-Elmer FT-IR spectrophotometer. 1H NMR and spectra were recorded on a Varian Gemini 400-MHz instrument and 13C NMR spectra on a Varian Mercury 400 100-MHz instrument in dimethylsulfoxide (DMSO-d6) as a solvent and tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in parts per million (δ ppm). Mass spectra were scanned on a Shimadzu LCMS 2010 spectrometer. In the conventional method, compounds were synthesized by using a random synthesizer. Bookie Rota vapor was used for distillation.

**Synthesis of N’-(1-(1H-Benzo[d]imidazol-2-yl)ethylidene)-2-cyanoacetohydrazide (1)**

N’-(1-(1H-Benzo[d]imidazol-2-yl)ethylidene)-2-cyanoacetohydrazide (1) was prepared according to the literature method.[32]

**Synthesis of 1-((1-(1H-Benzo[d]imidazol-2-yl)ethylidene)amino)-6-amino-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (3)**

A mixture containing N’-(1-(1H-benzo[d]imidazol-2-yl)ethylidene)-2-cyanoacetohydrazide (0.01 mole), Knoevenagel compound 2-benzylidenemalononitrile 2 (0.01 mol), and a catalytic amount of piperidine in ethanol (30 mL) was refluxed for 3 h. The mixture was then cooled down to room temperature and diluted with a few drops of water. The crystals formed were filtered, air dried, and recrystallized from aqueous DMF. Yield: 75%; mp 210–212 °C; IR (KBr, cm⁻¹): 3443 (NH₂), 3335
(-NH, benzimidazole), 3055 (C-H, aromatic), 2922 (C-H, CH₃), 2212 (CN), 1690 (CO), 1640 (C=C), 1558 (C=N); ¹H NMR (400 MHz, DMSO-d₆, ppm): δ = 1.32 (s, 3H, CH₃), 6.79–7.82 (m, 9H, Ar-H), 8.82 (s, 2H, NH₂), 12.81 (s, 1H, -NH benzimidazole); ¹³C NMR (100 MHz, DMSO-d₆, ppm): δ = 169.5, 160.2, 159.4, 155.8, 151.7, 134.9, 134.5, 132.8, 130.1, 128.7, 127.9, 123.2, 115.9, 115.5, 115.2, 76.6, 13.7; LCMS (ESI): m/z 393.13 [M⁺]. Anal. calcd. for (C₂₂H₁₅N₇O): C, 67.17; H, 3.84; N, 24.92%. Found: C, 65.34; H, 3.08; N, 23.51%.

**General Synthesis of 1-((1-(1H-Benzimidazol-2-yl)ethylidene) amino)-6-((arylidene)amino)-2-oxo-4-phenyl-1,2-dihydropyridine-3, 5-dicarbonitriles (4a-o)**

Intermediate 3 and different substituted aromatic aldehydes (0.01 mol) in ethanol (30 mL) were taken in a round-bottom flask and refluxed for 5 h. The separated solid was filtered, dried, and recrystallized from ethanol. All other compounds of this series were prepared using the same method.

1-((1-(1H-Benzimidazol-2-yl)ethylidene)amino)-6-((benzylidene)amino)-2-oxo-4-phenyl-1,2-dihydropyridine-3, 5-dicarbonitrile (4a)

Buff crystals (EtOH). Yield: 79%; mp 243–245 °C; IR (KBr, cm⁻¹): 3333 (-NH, benzimidazole), 3053 (C-H, aromatic), 2923 (C-H, CH₃), 2213 (CN), 1688 (CO), 1638 (C=C), 1560 (C=N); ¹H NMR (400 MHz, DMSO-d₆, ppm): δ = 1.30 (s, 3H, CH₃), 6.80–7.80 (m, 14H, Ar-H), 9.25 (s, 1H, CH=N), 12.8 (s, 1H, -NH benzimidazole); ¹³C NMR (100 MHz, DMSO-d₆, ppm): δ = 169.4, 163.8, 160.1, 155.7, 153.3, 151.6, 134.9, 133.8, 132.4, 131.1, 129.3, 128.9, 128.6, 127.2, 123.2, 115.9, 115.5, 115.2, 114.7, 13.6; LCMS (ESI): m/z 481.17 [M⁺]. Anal. calcd. for (C₂₉H₁₉N₇O): C, 72.34; H, 3.98; N, 20.36%. Found: C, 71.86; H, 3.26; N, 19.88%.

**CONCLUSION**

The synthesized compounds were screened for their in vitro antibacterial and antifungal activity. It may be concluded from Table 1 that structural and electronic diversity of these products affected their biological activities. We have found compounds 4b, 4e, 4h, and 4k to be the most distinctive derivatives identified in the present study because of their remarkable in vitro antimicrobial poteney. SAR studies revealed that when the ortho position is substituted by electron-withdrawing groups such as nitro and chloro, compounds exhibited antibacterial activity. Likewise when the ortho position is substituted by electron-donating groups such as hydroxy and methyl, compounds displayed antifungal activity. It may be considered a promising lead for further design and development of new lead molecules.

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SUPPLEMENTAL MATERIAL

Full experimental, antibacterial and antifungal assay, IR, $^1$H NMR, and mass spectral details for this article can be accessed on the publisher's website.

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