SYNTHESIS AND ANTIFUNGAL ACTIVITY OF BENZOXAZOLE DERIVATIVES WITH THEIR SAR ANALYSIS BY SAS-MAP

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Abstract

A simple and efficient method has been developed for the synthesis of benzoxazole derivatives. In the first step, 2-aminophenols reacted with various aldehydes in the presence of titanium supported nano-silica to produce an imine intermediate. Then the imine was oxidized to get the final azole compounds. Eleven derivatives were synthesized (*c*)-(*m*) *via* this simple and environmentally friendly procedure with high yields. The simple methodology (only 2 steps) with high yields for the reactions and easy procedure are the advantages of the newly developed method. The identification and characterization of all the synthesized compounds were confirmed by melting point, thin layer chromatography, FT-IR, ^{1}H NMR and ^{13}C NMR spectral data. Also elemental analysis was applied for five compounds. All the compounds were screened for antimicrobial activity by broth microdilution methods as recommended by CLSI. Of the tested compounds 2-(2,4-dichlorophenyl)-1,3-benzoxazole (*g*), and 2-(4-chlorophenyl)-1,3-benzoxazole (*f*) inhibited the growth of all examined fungi, while 2-(4-nitrophenyl)-1,3-benzoxazole (*c*) exhibited inhibitory activities only against the tested yeasts.

Rezumat

A fost dezvoltată o metodă simplă și eficientă pentru sinteza derivaților de benzoxazol. În prima etapă, 2-aminofenolii au reacționat cu diferite aldehide, în prezența nano-silicei cu titan pentru a produce un intermediar iminic. Ulterior imina a fost oxidată pentru a obține azoli finali. Au fost sintetizați cu randamente ridicate, prin această procedură simplă și ecologică, unsprezece compuși. Identificarea și caracterizarea tuturor compușilor sintetizați au fost confirmate prin: punctul de topire, cromatografie în strat subțire, FT-IR, ^{1}H RMN și ^{13}C RMN. De asemenea, analiza elementală a fost aplicată pentru cinci compuși. Toți compușii au fost analizați din punct de vedere microbiologic prin metode microdiluțiilor. Dintre compușii testați, 2-(2,4-diclorofenil)-1,3-benzoxazol (*g*) și 2-(4-clorfenil)-1,3-benzoxazol (*f*) au inhibat creșterea tuturor ciupercilor examinate, în timp ce 2-(4-nitrofenil)-1,3-benzoxazol (*c*) a prezentat activități inhibitoare numai împotriva drojdiilor testate.

Keywords: benzoxazole, synthesis, antifungal, SAR analysis, nano-TiCl$_4$.SiO$_2$

Introduction

Systemic fungal infections increased dramatically in the past few decades, especially in immune-compromised individuals suffering from tuberculosis, cancer, AIDS, and in organ transplant recipients [1]. The wide-spread use of antifungal drugs and their resistance against fungal infections have led to serious health concerns. Although different drugs such as novel azole compounds are available for the treatment of superficial and systemic mycoses, they are not completely effective in all cases. In addition, they all possess a certain degree of toxicity and quickly develop resistance due to the large-scale usage. Therefore, an urgent need for new antifungal chemical structures as alternative agents to the existing ones is required [2].

In this regard, the azole ring system is present in biologically active compounds which possess high antifungal properties.

In our previous reports, we described the preparation procedure for a number of imidazole, benzimidazole and benzotriazole derivatives with biological interest [3-6]. In the current study, we have synthesized some new derivatives of benzoxazoles as antifungal agents. Usually, nitrogen and sulphur containing organic compounds display a wide range of biological activities. For example benzoxazole heterocycles display a broad spectrum of biological activities such as hypoglycaemic [7], antiulcer [8], antifungal [9], anticonvulsant [10], antitumor [11] and anti-inflammatory [12] activities. Different protocols for the synthesis of benzoxazoles have been developed including copper-catalysed cyclizations of *o*-halobenzanilides or cross-coupling of primary amides [13, 14] and direct condensation of *o*-aminophenol with carboxylic acid or aldehyde [15]. In most cases, more than one step is required to synthesize these heterocycles. To avoid these limitations,

our study aimed towards the development of more efficient methods accompanied with higher yields for the synthesis of benzoxazoles in the presence of nano-TiCl$_4$.SiO$_2$. In our previous works we introduced titanium supported nano-silica as an environmental friendly and economical catalyst for organic reactions [16-21]. Easy handling, inexpensive, efficient, non-toxic, recoverable, and its reusability make this catalyst eco-friendly, synthetically acceptable and economically feasible. In the present study, we used this catalyst to synthesize our target compounds in a short period of time and with high yields in only two steps.

In order to compare the synthesized compounds based on theirs structures and activities, SAS (structure activity similarity) map analysis was performed. Furthermore, broth microdilution methods were used for screening the antifungal and antibacterial activities of synthetic compounds.

Materials and Methods

Chemistry

The chemicals were purchased from Merck and used without any additional purification. The products were characterized by FT-IR (ATR), ^{1}H-NMR, and a comparison of their physical properties with those reported in the literature was performed. FT-IR (ATR) spectra were acquired on a Bruker, Eqinox 55 spectrometer. A Bruker (DRX-400 Avance) NMR was used to record the ^{1}H NMR and ^{13}C NMR spectra. The
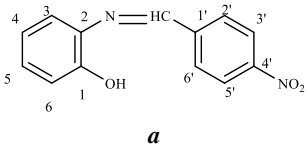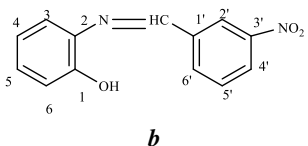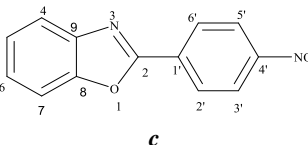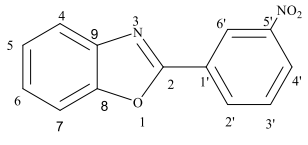
melting points were measured by a Buchi melting point B-540 apparatus. The elemental analysis was done by a Costech ECS 4010 CHNS-O analyser.

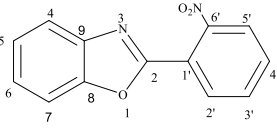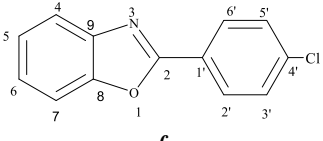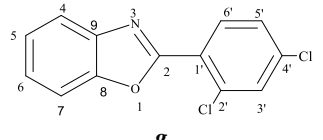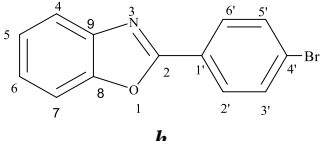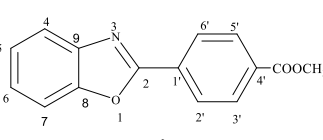*General procedure for synthesis of benzoxazole derivatives (**a**) - (**m**)*

A mixture of 2-aminophenol derivatives (1.5 mmol) and substituted aldehydes (1 mmol) with Ti supported nano-SiO$_2$ (0.1 g) in EtOH (2 mL) was stirred at room temperature for 1.5 h. Following completion of the reaction, the solvent was filtered and evaporated; small amounts of cold water were added and then dried. Our suggestion was that an imine intermediate is the product of this step. To make sure about the formation of this intermediate we isolated and identified the product of this step only for two compounds (intermediates (**a**) and (**b**) for compounds (**c**) and d respectively). Spectroscopic data confirmed our suggestion. Thus for other compounds (**e**) - (**m**) the reaction was performed *in situ* without any separation of the intermediate.

Subsequently, in the next step, by oxidation of the imine intermediate *via* KMnO$_4$ (1.7 mmol) and CH$_3$COOH (0.08 mL) in grinding, crude benzoxazole was formed. Purification of the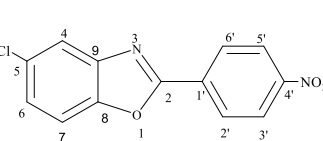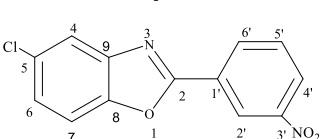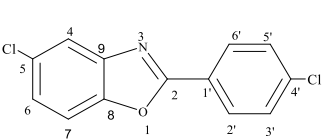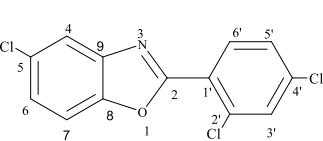 crude compound was carried out by recrystallization from acetone (solid products) or by chromatography using silica gel and mixtures of *n*-hexane/ethyl acetate (70/30) of increasing polarity.

Table I

Spectral data of benzoxazole derivatives

Compounds	Spectral data
a	Yield: 98%, Yellow Solid, Mp = 160 - 161°C; FT-IR, (ATR) = v̄: 3365 (O-H stretch), 1587 (C=N stretch), 1509 (NO$_2$ asym. stretch), 1480 (C=C stretch), 1335 (NO$_2$ sym. stretch), 1287 (C-N stretch), 1241 (C-O stretch), cm^{-1};^{1}H NMR (400 MHz, CDCl$_3$), σ(ppm) = 6.95 (t, J = 8 Hz, 1H$_6$), 7.06 (d, J = 8 Hz, 1H$_4$), 7.18 (brs, 1H, OH), 7.37 (d, J = 8 Hz, 2H$_{3,5}$), 8.10 (d, J = 8 Hz, 2H$_{2',6'}$), 8.37 (d, J = 8 Hz, 2H$_{3',5'}$), 8.8 (s, 1H, =CH); ^{13}C NMR (300 MHz, DMSO), σ(ppm) = 163.7(=C), 144.2 (C$_1$), 133.9(C$_{1'}$), 130.9(C$_5$), 127.4(C$_{6'}$), 127.3(C$_{2'}$), 123.1(C$_{5'}$), 121.7(C$_{3'}$), 120.3(C$_4$), 116.0 (C$_3$), 114.4 (C$_6$).
b	Yield: 86%, Yellow Solid, Mp = 136 - 137°C; FT-IR, (ATR) = v̄: 3345 (O-H stretch), 3046 (C-H stretch), 1630 (C=N stretch), 1583, 1476 (C=C stretch), 1513 (NO$_2$ asym. stretch), 1345 (NO$_2$ sym. stretch), 1286 (C-N stretch), 1239 (C-O stretch) cm^{-1}, ^{1}H NMR (400 MHz, Acetone), σ(ppm) = 6.8 - 7.0 (m, 1H$_6$, OH), 7.2 (t, 1H$_4$), 7. 5 (d, 1H$_3$), 7.85 (m, 1H$_5$), 8.3-8.4 (m, 2H$_{4',6'}$), 8.5 (d, 1H$_{5'}$), 8.8 (s, 1H$_{2'}$), 9.04 (s, 1H, =CH); ^{13}C NMR (300 MHz, DMSO), σ(ppm) = 164.9(=C), 145.4(C$_1$), 139.7(C$_{3'}$), 131.2(C$_{1'}$), 129.6(C$_{6'}$), 128.7(C$_{5'}$), 126.1(C$_5$), 131.2(C$_{4'}$), 123.1(C$_{2'}$), 122.4.0(C$_4$), 117.0(C$_3$), 116.1(C$_6$).
c	Yield: 92%, Brown Solid, Mp = 262 - 264°C [266 - 268°C] [25]; FT-IR, (ATR) = v̄: 3112 (C-H stretch), 1598 (C=N stretch), 1555, 1451 (C=C stretch), 1519 (NO$_2$, asym. stretch), 1348 (NO$_2$, sym. stretch) cm^{-1}, ^{1}H NMR (400 MHz, CDCl$_3$), σ(ppm) = 7.26-8.1 (m, 4H$_{4,5,6,7}$), 8.44 (brs, 4H$_{6',5',3',2'}$); ^{13}C NMR (300 MHz, DMSO), σ(ppm) = 145.9(C$_2$), 133.0(C$_{4'}$), 132.9(C$_{1'}$), 129.2(C$_{6'}$), 129.1(C$_{2'}$), 124.1(C$_{5'}$), 123.0(C$_{3'}$), 117.0(C$_5$), 116.2(C$_6$), 116.0(C$_4$), 115.7(C$_7$) ppm.
d	Yield: 87%, Gray Solid, Mp = 210 - 212°C [211 - 212°C] [25]; FT-IR, (ATR) = v̄: 3097 (C-H stretch), 1613 (C=N stretch), 1525 (NO$_2$, asym. stretch), 1474, 1453 (C=C stretch), 1351 (NO$_2$, asym. stretch), 1241 (C-O stretch) cm^{-1}, ^{1}H NMR (400 MHz, Acetone), σ(ppm) = 7.48 - 8.98 (m, 8H$_{4,5,6,7,6',4',3',2'}$); ^{13}C NMR (300 MHz, DMSO), σ(ppm) = 144.2(C$_2$), 133.9(C$_{5'}$), 130.9(C$_{2'}$), 127.4(C$_{3'}$), 127.3(C$_5$), 123.1(C$_6$), 121.7(C$_{4'}$), 116.0(C$_4$), 114.4(C$_7$).

Compounds	Spectral data
e	Yield: 90%, Brown Solid, Mp = 104 - 105°C [101 - 103°C] [25]; FT-IR, (ATR) = ῡ: 1620 (C=N stretch), 1534 (NO$_2$, asym. stretch), 1452 (C=C stretch), 1349 (NO$_2$, sym. stretch), 1240 (C-O stretch) cm^{-1}, ^{1}H NMR (400 MHz, CDCl$_3$), σ(ppm) = 7 - 8 (m, 8H$_{4,5,6,7,5',4',3',2'}$); ^{13}C NMR (300 MHz, DMSO), σ(ppm) = 145.4(C$_2$), 133.0(C$_{6'}$), 132.9(C$_{1'}$), 129.2(C$_{3'}$), 129.1(C$_{4'}$), 128.7(C$_{2'}$), 124.1(C$_5$), 123.0(C$_6$), 116.0(C$_4$), 115.7(C$_7$); C$_{13}$H$_8$N$_2$O$_3$, Calculated: C, 65.00; H, 3.36; N, 11.66. Found: C, 64.6; H, 3.39; N, 11.67%.
f	Yield: 85%, Brown Solid, Mp = 145 - 147 °C [148 - 150°C] [24]; FT-IR, (ATR) = ῡ: 3060 (C-H stretch), 1617 (C=N stretch), 1574, 1483 (C=C stretch), 1344 (C-N stretch), 1244 (C-O stretch), 1091 (C-Cl stretch) cm^{-1}, ^{1}H NMR (400 MHz, Acetone), σ(ppm) = 7.44 - 8.26 (m, 8H$_{4,5,6,7,5',6',3',2'}$); ^{13}C NMR (300 MHz, DMSO), σ(ppm) = 145.4(C$_2$), 136.9(C$_{4'}$), 133.7(C$_{1'}$), 129.6(C$_{6'}$), 129.1(C$_{2'}$), 128.8(C$_{5'}$), 127.6(C$_{3'}$), 127.0(C$_5$), 124.1(C$_6$), 123.0(C$_4$), 116.3(C$_7$).
g	Yield: 88%, Gray Solid, Mp = 141 - 142°C [144 - 145°C] [24]; FT-IR, (ATR) = ῡ: 3066 (C-H stretch), 1590 (C=N stretch), 1560, 1471 (C=C stretch), 1375 (C-N stretch), 1191 (C-O stretch), 1027 (C-Cl stretch) cm^{-1}, ^{1}H NMR (400 MHz, Acetone), σ(ppm) = 7.45 (brs, 2H$_{4,7}$), 7.7 (brs, 1H$_{5'}$), 7.8 (m, 3H$_{6',5,6}$), 8.25 (brs, 1H$_{3'}$); ^{13}C NMR (300 MHz, DMSO), σ(ppm) = 145.3(C$_2$), 135.4(C$_{4'}$), 128.8(C$_{2'}$), 128.7(C$_{1'}$), 127.4(C$_{6'}$), 126.6(C$_{3'}$), 124.0(C$_5$), 122.8(C$_6$), 116.9(C$_4$), 115.7(C$_7$).
h	Yield: 91%, Gray Solid, Mp = 154 - 156°C [157 - 158°C] [24]; FT-IR, (ATR) = ῡ: 3057(C-H stretch), 1616 (C=N stretch), 1592, 1483 (C=C stretch), 1294 (C-N stretch), 1243 (C-O stretch), 1068 (C-Br stretch) cm^{-1}, ^{1}H NMR (400 MHz, DMSO-d_6), σ(ppm) = 7.88 (brs, 2H$_{4,7}$), 8.24 (brs, 4H$_{5',6',3',2'}$), 8.62 (brs, 2H$_{5,6}$); ^{13}C NMR (300 MHz, DMSO), σ(ppm) = 144.4(C$_2$), 132.6(C$_{5'}$), 132.5(C$_{3'}$), 130.0(C$_{1'}$), 129.2(C$_{2'}$), 129.1(C$_{6'}$), 126.7(C$_5$), 123.6(C$_{4'}$), 118.4(C$_6$), 116.1(C$_4$), 115.6(C$_7$).
i	Yield: 68%, Gray Solid, Mp = 169 - 171°C; FT-IR, (ATR) = ῡ: 3163 (C-H stretch), 2955 (C-H stretch), 1726 (C=O stretch), 1605 (C=N stretch), 1557, 1454 (C=C stretch), 1276 (C-O stretch) cm^{-1}, ^{1}H NMR (400 MHz, DMSO-d_6), σ(ppm) = 4.35 (s, 3H, CH$_3$), 7.92 (brs, 2H$_{5,6}$), 8.23 (brs, 2H$_{4,7}$), 8.71 (brs, 4H$_{5',6',3',2'}$); ^{13}CNMR (300 MHz, DMSO), σ(ppm) = 164.9(C=O), 145.4(C$_2$), 133.7(C$_9$), 131.2(C$_{1'}$), 130.6(C$_{4'}$), 129.8(C$_{5'}$), 128.7(C$_{3'}$), 126.1(C$_{5'}$), 124.2(C$_{2'}$), 123.1(C$_5$), 122.4(C$_{6'}$), 117.0(C$_4$), 116.1(C$_7$), 43.4(CH$_3$); C$_{15}$H$_{11}$NO$_3$, Calculated: C, 71.14; H, 4.38; N, 5.53. Found: C, 70.64; H, 4.42; N, 5.89 %.
j	Yield: 83%, Yellow Solid, Mp = 220 - 222°C; FT-IR, (ATR) = ῡ: 3093 (C-H stretch), 1602 (C=N stretch), 1552, 1453 (C=C stretch), 1519 (NO$_2$, asym. stretch), 1348 (NO$_2$, sym. stretch), 1285 (C-O stretch), 1063 (C-Cl stretch) cm^{-1}, ^{1}H NMR (400 MHz, Acetone), σ(ppm) = 7.5 (brs, 1H$_6$), 7.8 - 8.0 (m, 2H$_{4,7}$), 8.49 (brs, 4H$_{5',6',3',2'}$); ^{13}CNMR (300 MHz, DMSO), σ(ppm) = 145.4(C$_2$), 128.7(C$_{6'}$), 123.8(C$_{5'}$), 123.1(C$_6$), 117.0(C$_4$), 115.7(C$_7$); C$_{13}$H$_7$ClN$_2$O$_3$, Calculated: C, 56.85; H, 2.57; N, 10.20. Found: C, 56.75; H, 2.62; N, 10.25 %.
k	Yield: 79%, Gray Solid, Mp = 123 - 125°C; FT-IR, (ATR) = ῡ: 3093 (C-H stretch), 1621 (C=N stretch), 1527 (NO$_2$, asym. stretch), 1449 (C=C stretch), 1353 (NO$_2$, sym. stretch), 1195 (C-O stretch), 1101 (C-Cl stretch) cm^{-1}, ^{1}H NMR (400 MHz, Acetone), σ(ppm) = 7.9 (brs, 1H$_6$), 7.8 (brs, 1H$_7$), 8.0 (brs, 2H$_{5',4}$), 8.5 (brs, 1H$_{4'}$), 8.77 (brs, 1H$_{6'}$), 9.0 (brs, 1H$_{2'}$); ^{13}CNMR (300 MHz, DMSO), σ(ppm) = 145.4(C$_2$), 128.7(C$_{6'}$), 123.8(C$_{2'}$), 123.2(C$_6$), 117.0(C$_4$), 115.8(C$_7$).
l	Yield: 81%, Gray Solid, Mp = 177 - 179°C; FT-IR, (ATR) = ῡ: 3066 (C-H stretch), 1611 (C=N stretch), 1550, 1481 (C=C stretch), 1332 (C-N stretch), 1261 (C-O stretch), 1089 (C-Cl stretch) cm^{-1}, ^{1}H NMR (400 MHz, Acetone), σ(ppm) = 7.45 (brs, 1H$_6$), 7.6-7.8 (m, 4H$_{5',6',3',2'}$), 8.25 (brs, 2H$_{4,7}$); ^{13}CNMR (300 MHz, DMSO), σ(ppm) = 145.4(C$_2$), 128.7(C$_{5'}$), 123.8(C$_{2'}$), 123.1(C$_6$), 117.0(C$_4$), 115.8(C$_7$); C$_{13}$H$_7$Cl$_2$NO, Calculated: C, 59.12; H, 2.67; N, 5.30. Found: C, 58.92; H, 2.45; N, 5.72 %.
m	Yield: 76%, Gray Solid, Mp = 148 - 150°C; FT-IR, (ATR) = ῡ: 3092 (C-H stretch), 1586 (C=N stretch), 1561, 1448 (C=C stretch), 1388 (C-N stretch), 1257 (C-O stretch), 1092 (C-Cl stretch) cm^{-1}, ^{1}H NMR (400 MHz, CDCl$_3$), σ(ppm) = 7.01 - 7.12 (m, 2H$_{6,5'}$), 7.4 - 7.5 (m, 2H$_{6',3'}$), 7.81 (brs, 1H$_7$), 8.25 (brs, 1H$_4$); ^{13}CNMR (300 MHz, DMSO), σ(ppm) = 145.4(C$_2$), 128.7(C$_{6'}$), 123.8(C$_{5'}$), 123.1(C$_6$), 117.0(C$_4$), 115.7(C$_7$); C$_{13}$H$_6$Cl$_3$NO, Calculated: C, 52.30; H, 2.03; N, 4.69. Found: C, 52.15; H, 2.39; N, 4.48 %.

Biological activity

Microorganisms. The antifungal activities of the synthetic compounds against some standard strains of fungi, including *Candida ablicans* (ATCC 10261, 1905, 2730, 1912), *C. tropicalis* (ATCC 4344, 750), *C. krusei* (ATCC 6258), *C. glabrata* (ATCC 90030, 863, 2192), *C. dubliniensis* (ATCC 8500, 8501, 7988, 7987), *C. neoformance* (ATCC 9011), *Aspergillusflavus* (ATCC 64025), *A. clavatus* (CBS 514.65), *A. fumigatus* (ATCC 14110) and *Exophiala* dermatitidis (ATCC

109136) were determined. In addition, the antifungal activities of the compounds were tested against six clinical isolates of yeasts identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and three clinical isolates of dermatophytes (*Epidermophyton floccosum*, *Microsporum canis* and *Trichophyton rubrum*) identified by both morphological and molecular methods [26]. The antifungal susceptibility of the tested yeasts and *Aspergillus* species against fluconazole (Sigma, St. Louis, MO, USA) and dermatophytes against griseofulvin (Sigma) were examined by microdilution methods [27, 28].

Determination of minimum inhibitory concentration.
MICs were determined by using the broth micro-dilution method recommended by the CLSI (Clinical & Laboratory Standards Institute) with some modifications. In order to determinate the antimicrobial activities against fungi, serial dilutions of the synthetic compounds (1 - 1024 µg/mL) were prepared in 96-well microtiter plates using Roswell Park Memorial Institute medium (RPMI)-1640 media (Sigma, St. Louis, MO, USA) buffered with 3-(N-morpholino)propanesulfonic acid (MOPS) (Sigma). Stock inoculums were prepared by suspending three colonies of the examined yeast in 5 mL sterile 0.85% NaCl, and adjusting the turbidity of the inoculums to 0.5 McFarland standards at 530 nm wavelengths (this yields stock suspension of $1 - 5 \times 10^6$ cells/mL). For moulds (*Aspergillus* spp. and dermatophytes), conidia were recovered from the 7-day old cultures grown on potato dextrose agar by a wetting loop with tween-20. The collected conidia were transferred in sterile saline and their turbidity was adjusted to OD = 0.09 - 0.11 that yields $0.4 - 5 \times 10^6$ conidia/mL. The working suspension was prepared by making a 1/50 and 1/1000 dilution with RPMI of the stock suspension for moulds and yeasts, respectively. Working inoculums (0.1 mL) were added to the microtiter plates, which were incubated in a humid environment at 30ºC for 24 - 48 h. Uninoculated medium (200 µL) was included as a sterility control. In addition, growth controls (medium with inoculums but without antibiotics or the synthetic compounds) were also included.

The growth in each well was compared with that of the growth in the control well. MICs were visually determined and defined as the lowest concentration of the compounds produced ≥ 95% growth reduction compared with the growth in the control well. Each experiment was performed in triplicate.

In addition, media from the wells with fungi showing no visible growth were further cultured on Sabouraud dextrose agar (Merck, Darmstadt, Germany) to determine the minimum fungicidal concentration (MFC). MFCs were determined as the lowest concentration yielding no more than 4 colonies, which resulted in mortality of 98% of the microbes in the initial inoculums.

SAS map analysis
To perform SAS analysis, the structures of the synthesized compounds were generated using Marvin-

Sketch (Marvin 5.7, 2014, ChemAxon). Subsequently, *Open Babel 2.3.2* was used to calculate the MACCS fingerprints of all compounds. The resulted fingerprints were then entered in our *in house* application implemented in *Visual.NET*. The pairwise tanimoto distance calculation of compounds was done using the following equation:

$$T_d = \frac{c}{a+b-c}, \qquad (1)$$

where (a) and (b) represent the number of bit sets in fingerprint of compared compounds and c denotes the number of common bits [29].

Meanwhile, activity similarities were calculated according to the bellow equation.

$$sim_{activity}(i,j) = 1 - \left(\frac{[|activity_i - activity_j|]}{[activity_{max} - activity_{min}]} \right), \qquad (2)$$

where the two symbols activity max and activity min represent the MIC90 values for the most active and the least potent compounds, respectively [30]. Finally, the visualization of structure activity analysis was performed using a 2D plot.

Results and Discussion

Therefore, to optimize the reaction conditions we repeated the reactions in different situations in the point of solvents, catalysts and oxidizing agents (Table II). We have investigated the synthesis of *N*-2-hydroxyphenyl-4-nitrophenyldimine *(a) via* the condensation of 4-nitrobenzaldehyde (1 mmol) and 2-aminophenol (1.5 mmol) with and without the nano-TiCl₄.SiO₂. We observed that the reaction did not continue in the absence of this catalyst (Table II, entry 1). Also we repeated the reaction with different amounts of the catalyst and we found that the most appropriate amount for catalyst was 0.1 g that gave 2-(4-nitrobenzylideneamino) phenol (imine intermediate) with 97% yield in 1 h (Table II, entry 4). On the other hand, we tried to repeat the reaction in different solvents such as ethanol, ethylacetate and chloroform (Table II, Entry 3, 6 and 7 respectively). Our results showed that ethanol was the best solvent for this type of reactions (Table II, entry 3). In the next step, for oxidizing the imine intermediate to 2-(4-Nitrophenyl)-1,3-benzoxazole *(c)* various reagents were also tested, for example ceric ammonium nitrate (CAN), *p*-chloranil and aerobic oxidation (Table II, entry 9, 10 and 11), but according to our experiments the KMnO₄ along with CH₃COOH by the molar ratio, 1.7:1.4 for KMnO₄ and CH₃COOH respectively was the BEST oxidation agent *via* grinding for 1 minute (Table II, entry 4).

This procedure for synthesis of benzoxazole was compared with different methods from literature (Table II, entry 12 and 13). Finally 2-aminophenol and its 4-chloro analogue reacted with various aldehydes in the optimized condition as substrates for the synthesis of benzoxazole derivatives and the results being presented in Table III.

Table II

Synthesis of N-2-hydroxyphenyl-4-nitrophenyldimine (*a*) and 2-(4-nitrophenyl)benzo[d]oxazole (*c*) under various conditions

Entry	Catalyst (g)	Oxidant	Solvent	Condition	Time (h)	Yield (%)	Ref.
1	No catalyst	KMnO$_4$/CH$_3$COOH	-	RT	4	0	-
2	Nano-TiCl$_4$.SiO$_2$ (0.15)	KMnO$_4$/CH$_3$COOH	-	RT	3	57	-
3	Nano-TiCl$_4$.SiO$_2$ (0.15)	KMnO$_4$/CH$_3$COOH	EtOH	RT	1	92	-
4	Nano-TiCl$_4$.SiO$_2$ (0.1)	KMnO$_4$/CH$_3$COOH	EtOH	RT	1	97	-
5	Nano-TiCl$_4$.SiO$_2$ (0.05)	KMnO$_4$/CH$_3$COOH	EtOH	RT	3	85	-
6	Nano-TiCl$_4$.SiO$_2$ (0.1)	KMnO$_4$/ CH$_3$COOH	EtOAc	RT	1	70	-
7	Nano-TiCl$_4$.SiO$_2$ (0.1)	KMnO$_4$/CH$_3$COOH	CH$_3$Cl	RT	3	66	-
8	Nano-TiCl$_4$.SiO$_2$ (0.1)	KMnO$_4$/CH$_3$COOH	EtOH	Reflux	1	90	-
9	Nano-TiCl$_4$.SiO$_2$ (0.1)	CAN	EtOH	RT	1	72	-
10	Nano-TiCl$_4$.SiO$_2$ (0.1)	*p*-chloranil	EtOH	RT	1	80	-
11	Nano-TiCl$_4$.SiO$_2$ (0.1)	air	EtOH	RT	24	50	-
12	Ni-SiO$_2$	-	EtOH	RT	1.5	90	[22]
13	Molecular Iodine	-	-	MW	10 min	90	[23]

(a) The molar ratio of 2-aminophenol:4-nitrobenzaldehyde is 1.5:1; RT = room temperature, MW = microwave

Table III

Preparation of various benzoxazoles in the presence of nano-TiCl$_4$.SiO$_2$[a]
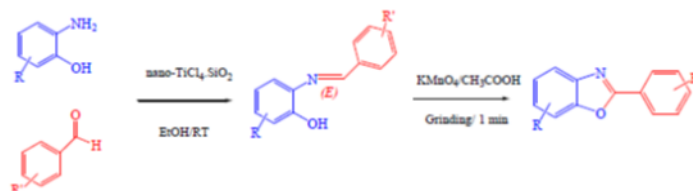
Entry	R	R´	Products	yield (%)[b]	Time (h)
c	H	4-NO2		92	1
d	H	3-NO2		87	1
e	H	2-NO2		90	1
f	H	4-Cl		85	1.5
g	H	2,4-Cl		88	1.5
h	H	4-Br		91	1.5
i	H	4-COOCH3		68	2.5
j	4-Cl	4-NO2		83	2
k	4-Cl	3-NO2		79	2
l	4-Cl	4-Cl		81	2
m	4-Cl	2,4-Cl		76	2

Reaction conditions: 2-aminophenol (1.5 mmol), substituted aldehydes (1 mmol), nano-TiCl$_4$.SiO$_2$(0.1 g), RT, EtOH. b) Isolated yields

Table IVa

Minimum inhibitory and fungicidal concentrations of the synthetic compounds (µg/mL) against the examined fungi

	Microorganism	(c) MIC 50	(c) MIC 90	(c) MFC	(d) MIC 50	(d) MIC 90	(d) MFC	(e) MIC 50	(e) MIC 90	(e) MFC	(f) MIC 50	(f) MIC 90	(f) MFC	(g) MIC 50	(g) MIC 90	(g) MFC	(h) MIC 50	(h) MIC 90	(h) MFC
Yeasts	C. albicans (ATCC 10261)	16	32	512	512	512	512	512	512	512	8	32	512	8	16	512	128	256	512
	C. albicans (ATCC 1905)	64	128	512	512	512	512	256	512	512	16	32	512	32	64	512	256	512	512
	C. albicans (ATCC 2730)	16	32	512	256	512	512	128	256	512	16	32	512	16	32	512	64	128	512
	C. albicans (ATCC 1912)	16	32	512	16	32	512	16	32	512	8	16	512	16	32	512	16	32	512
	C. dubliniensis (ATCC 8500)	16	32	512	512	512	512	512	512	512	4	8	512	32	64	512	256	512	512
	C. dubliniensis (ATCC 8501)	32	64	512	128	256	512	256	512	512	32	64	512	64	128	512	256	512	512
	C. dubliniensis (ATCC 7988)	32	64	512	512	512	512	512	512	512	64	128	512	32	64	512	64	128	512
	C. dubliniensis (ATCC 7987)	16	32	512	128	256	512	128	256	512	16	32	512	32	64	512	32	64	512
	C. glabrata (ATCC 90030)	2	4	512	8	16	512	512	512	512	1	2	512	2	4	512	16	32	512
	C. glabrata (ATCC 863)	1	2	512	2	4	512	512	512	512	0.25	0.5	512	0.5	1	512	1	2	512
	C. glabrata (ATCC 2192)	2	4	512	8	16	512	64	128	512	1	2	512	4	8	512	8	16	512
	C. krusei (ATCC 6258) *	32	64	512	128	256	512	256	512	512	32	64	512	64	128	512	128	256	512
	C. parapilopsis (ATCC 4344)	32	64	512	512	512	512	128	256	512	32	64	512	64	128	512	128	256	512
	C. tropicalis (ATCC 750)	64	128	512	128	256	512	64	128	512	32	64	512	32	64	512	128	256	512
	C. tropicalis (SUMSCC 194)*	128	256	512	512	512	512	512	512	512	64	128	512	128	256	512	512	512	512
	C. albicans (SUMSCC 239)	8	16	512	512	512	512	512	512	512	8	16	512	8	16	512	512	512	512
	C. tropicalis (SUMSCC 611) *	128	256	512	512	512	512	512	512	512	32	64	512	128	256	512	512	512	512
	C. tropicalis (SUMSCC 203)	128	256	512	512	512	512	512	512	512	32	64	512	64	128	512	512	512	512
	C. tropicalis (SUMSCC 2303) *	128	256	512	512	512	512	512	512	512	32	64	512	128	256	512	512	512	512
	C. tropicalis (SUMSCC 625) *	16	32	512	512	512	512	512	512	512	4	8	512	16	32	512	512	512	512
	C. neoformans (ATCC 9011)	64	128	512	512	512	512	512	512	512	32	64	512	64	128	512	128	256	512
Filamentous fungi	A. fumigatus (ATCC 14110)	512	512	512	512	512	512	512	512	512	64	128	512	64	128	512	512	512	512
	A. flavus (ATCC 64025)	512	512	512	512	512	512	512	512	512	128	256	512	128	512	512	512	512	512
	A. clavatus (CBS 514.65)	512	512	512	512	512	512	512	512	512	64	128	512	64	128	512	512	512	512
	M. canis (clinical isolate)	512	512	512	512	512	512	512	512	512	64	128	512	4	8	512	512	512	512
	T. rubrum (clinical isolate)	8	16	512	512	512	512	512	512	512	64	64	512	8	16	512	512	512	512
	E. flucusom (clinical isolate)	512	512	512	512	512	512	512	512	512	32	64	256	8	16	64	512	512	512
	E. dermatitidis (ATCC 109136)	512	512	512	512	512	512	512	512	512	64	128	512	8	16	512	512	512	512

*Azole-resistant strain; MIC: Minimum inhibitory concentration, MFC: Minimum fungicidal concentration

Table IVb

Minimum inhibitory and fungicidal concentrations of the synthetic compounds (µg/mL) against the examined fungi

	Microorganism	(i) MIC 50	(i) MIC 90	(i) MFC	(j) MIC 50	(j) MIC 90	(j) MFC	(k) MIC 50	(k) MIC 90	(k) MFC	(l) MIC 50	(l) MIC 90	(l) MFC	(m) MIC 50	(m) MIC 90	(m) MFC	PC MIC 50	PC MIC 90	PC MFC
Yeasts	C. albicans (ATCC 10261)	512	512	512	64	128	512	64	128	512	512	512	512	128	256	512	2	8	512
	C. albicans (ATCC 1905)	512	512	512	512	512	512	64	128	512	512	512	512	256	512	512	0.25	0.5	64
	C. albicans (ATCC 2730)	256	512	512	32	64	512	32	64	512	256	512	512	16	32	512	0.5	1	2
	C. albicans (ATCC 1912)	128	256	512	16	32	512	16	32	512	32	64	512	16	32	512	0.5	1	64
	C. dubliniensis (ATCC 8500)	512	512	512	64	256	512	256	512	512	512	512	512	256	512	512	0.25	0.5	64
	C. dubliniensis (ATCC 8501)	512	512	512	64	128	512	64	128	512	128	256	512	64	128	512	0.5	1	32
	C. dubliniensis (ATCC 7988)	512	512	512	256	512	512	128	256	512	512	512	512	256	256	512	0.5	1	4
	C. dubliniensis (ATCC 7987)	512	512	512	64	128	512	64	128	512	64	128	512	32	64	512	0.5	1	4
	C. glabrata (ATCC 90030)	512	512	512	8	16	512	8	16	512	512	512	512	512	512	512	2	4	512
	C. glabrata (ATCC 863)	512	512	512	2	8	512	0.25	0.5	512	512	512	512	512	512	512	0.5	1	16
	C. glabrata (ATCC 2192)	512	512	512	8	16	512	8	16	512	256	512	512	256	512	512	0.25	0.5	32
	C. krusei (ATCC 6258) *	512	512	512	128	256	512	256	512	512	512	512	512	128	256	512	32	128	512
	C. parapilopsis (ATCC 4344)	512	512	512	32	64	512	32	64	512	128	256	512	32	64	512	1	2	16
	C. tropicalis (ATCC 750)	128	256	512	64	128	512	128	256	512	64	128	512	64	128	512	16	32	512
	C. tropicalis (SUMSCC 194)*	512	512	512	128	256	512	256	512	512	512	512	512	256	512	512	128	256	512
	C. albicans (SUMSCC 239)	512	512	512	256	512	512	512	512	512	256	512	512	512	512	512	0.12	0.25	512
	C. tropicalis (SUMSCC 611) *	512	512	512	512	512	512	512	512	512	512	512	512	512	512	512	128	256	512
	C. tropicalis (SUMSCC 203)	512	512	512	512	512	512	256	512	512	512	512	512	256	512	512	4	8	512
	C. tropicalis (SUMSCC 2303) *	512	512	512	128	256	512	128	256	512	512	512	512	128	256	512	128	256	512
	C. tropicalis (SUMSCC 625) *	512	512	512	512	512	512	512	512	512	512	512	512	256	512	512	128	256	512
	C. neoformans (ATCC 9011)	512	512	512	512	512	512	32	64	512	512	512	512	128	256	512	0.5	1	512
Filamentous fungi	A. fumigatus (ATCC 14110)	512	512	512	512	512	512	512	512	512	512	512	512	512	512	512	2	4	512
	A. flavus (ATCC 64025)	512	512	512	512	512	512	512	512	512	512	512	512	512	512	512	0.5	1	512
	A. clavatus (CBS 514.65)	512	512	512	512	512	512	512	512	512	512	512	512	512	512	512	2	4	512
	M. canis (clinical isolate)	256	512	512	256	512	512	256	512	512	128	512	512	128	256	512	0.5	1	512
	T. rubrum (clinical isolate)	512	512	512	512	512	512	512	512	512	256	512	512	512	512	512	1	2	512
	E. flucusom (clinical isolate)	512	512	512	512	512	512	256	512	512	512	512	512	256	512	512	0.5	1	512
	E. dermatitidis (ATCC 109136)	512	512	512	512	512	512	512	512	512	512	512	512	256	512	512	8	16	32

*Azole-resistant strain; MIC: Minimum inhibitory concentration, MFC: Minimum fungicidal concentration

Antifungal activities of the synthetic compounds
Tables IVa and IVb summarizes the inhibitory activities of the synthetic compounds and control drugs against the tested fungi. By comparing MIC values of the synthetic compounds, (*g*) and (*f*) both exhibited strong inhibitory activities against all of the tested fungi including both yeasts and filamentous fungi with MICs GM (geometric means) of 36.2 µg/mL and 46.3 µg/mL, respectively. Furthermore, (*c*) inhibited the growth of the both susceptible and resistant strains of *Candida* at concentrations ranging from 2 µg/mL to 256 µg/mL. Although, (*k*) and (*j*) showed no antifungal activities against the examined filamentous fungi, they both inhibited the growth of the tested yeasts, except those of azole-resistant strains. Of the synthetic compound, (*f*), (*k*), (*c*), (*g*) and (*m*) completely inhibited the growth of *Crytococccus neoformance* at concentrations ranging from 64 µg/mL to 256 µg/mL.

In comparison with the antifungal activities of the synthetic compounds based on variation of substitutions on 2,3 and 4-position of phenyl ring, we found that the compounds (*f*) and (*g*) with Cl residue in *para* and *ortho*-positions of phenyl ring respectively exhibited better antifungal activities against the tested fungi than the other compounds. In comparison to these mentioned compounds, addition of another Cl residue to the 5-position of benzoxazole ring resulted respectively (*l*) and (*m*) compounds with considerable lower anti-fungal activities. Replacement of Cl with NO_2 residue in 4-position of phenyl ring of (*c*) reduced its anti-fungal activity compared to (*f*).

Similarly, the addition of Cl residue to the 5-position of benzoxazole ring in (*j*) reduced its antifungal activity

compared to (*c*). Moreover, exchanging of nitro group from *para*-position in c to *ortho* or *meta*-positions in (*e*) and (*d*) considerably decreased its activity on *Candida sp.* Of the examined synthetic compounds (*f*), (*g*) and (*c*) all were effective against azole-resistant strains *of Candida* at concentrations ranging from 8 - 256 µg/mL, suggesting that the modes of action of this compound are different from the examined antibiotics.

Map analysis

The SAS analysis for all species was performed separately. For example the SAS map for *Candida albicans* is depicted in Figure 1.
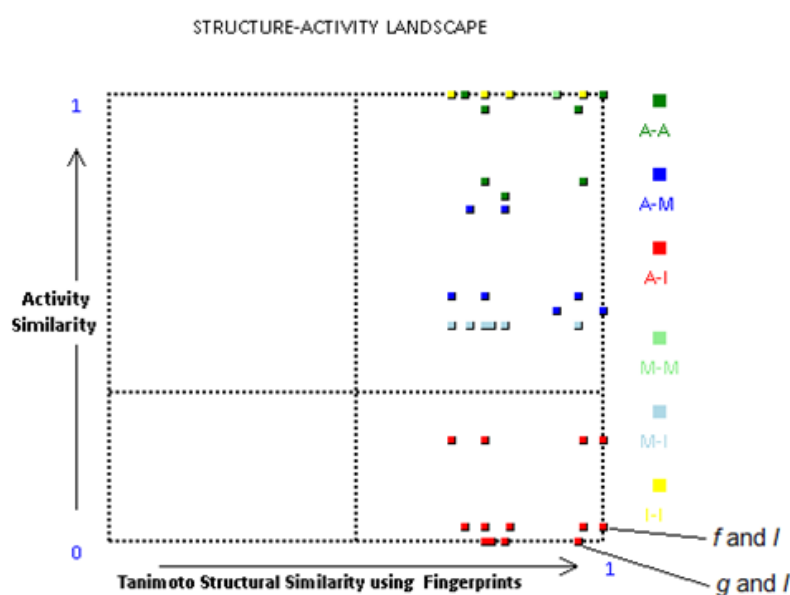
Figure 1.

A plot of SAS-map for *C. albicans* ; where red dots depict "Cliffs" , or points which indicate a little difference in structure, but a great difference in activity between the two synthesized substance

As seen in Figure, the SAS plot can be divided into four regions. The bottom right area is presenting the pairwise comparisons of the compounds with similar structures but dissimilar activity values (activity cliffs). As an example an outstanding activity cliff in this series of compounds refers to compounds (*f*) and (*l*) wherein a chlorine atom was inserted at *meta*-position of benzoxazole ring. This modification led to a significant drop in the activity of the resulted compound ((*f*), MIC_{50} = 8, and in case of (*l*) MIC_{50} > 256). Also as shown in Figure 1 the two compounds (*l*) and (*g*) are different considering a chlorine atom which is in the 2-phenyl position in compound (*g*) instead of *meta*-position of benzoxazole in compound (*l*). This difference caused such dissimilarity in effect so that the MIC_{50} value of compound (*l*) is more than 256 but for compound (*g*) is 8.

Conclusions

In conclusion, we have developed a novel and highly efficient method for the synthesis of benzoxazoles by treatment of 2-aminophenol and substituted aldehydes in the presence of Ni supported silica as an effective Lewis acid. This methodology may find widespread uses in organic synthesis for preparation of the benzoxazoles. In the present study, some of the synthetic compounds including (*f*) and (*g*) exhibited a great activity against tested *Aspergillus*, *Candida* and dermatophytes. Comparing the structure and activity of these two compounds with the others, revealed that the presence of Cl residue in *para* and *ortho*-position of phenyl ring, enhance the antifungal activity. Altogether, regarding the broad spectrum antifungal activities of some of the tested compounds even against azole resistant strains, they might be good candidates

for further *in vivo* studies to elucidate their effects and toxicity as novel antifungal drugs.

Conflict of interest
The authors declare no conflict of interest.

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