



Synthesis, Characterization, Crystal Structure and Free Radical Scavenging Activities of 2-(2-Benzylamine)-6-[(2-benzylamine)amino]-5-nitro-1H-benzo[de]-isoquinoline-1,3(2H)-dione

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2-(2-Benzylamine)-6-[(2-benzylamine)amino]-5-nitro-1H-benzo[de]-isoquinoline-1,3(2H)-dione (BBNID, **3**) was synthesized and then characterized by FT-IR, NMR and elemental analysis. The crystal structure of BBNID was investigated using X-ray diffraction and SHELXTL97 software and the result indicated that BBNID crystallized in the monoclinic system, space group C2/c with $a = 8.8032$ (14), $b = 8.822$ (2), $c = 13.994$ (4) Å, $V = 1005.9$ (4) Å³; $Z = 2$. The free radical scavenging activity screening results showed that BBNID exhibited better scavenging activity than the commercial antioxidant BHT against 2,2-diphenyl-1-picrylhydrazyl radical (DPPH[•]), with IC₅₀ of 43.10 µM.

Keywords: Naphthalimides, Synthesis, Crystal, Free radical scavenging activity.

INTRODUCTION

The significance of free radicals and reactive oxygen species in the pathogenesis of multifarious diseases has attracted a great deal of chemist's interest¹⁻³. Free radical scavenger are now fabricated as the drug candidates to counter many diseases, including carcinogenesis, inflammation, atherogenesis and aging in aerobic organisms^{4,5}. So recently, there has been considerable investment in efforts to develop efficient new free radical scavenging agents.

Naphthalimides, which are characterized by the presence of a coplanar chromophore and, usually, a π -deficient aromatic system, as well as one or two basic side chains, constitute an important class of prodrugs in anticancer therapy^{6,7}. They display high levels of antitumor activity toward multifarious murine and human tumor cells^{8,9}. Two members of this class of compounds, amonifide and mitonafide, are undergoing clinical trials⁹. Previous work demonstrated that good antitumor activity may lead to good free radical activity or antioxidant activity. It is thus to expect that naphthalimides may possess potential free radical activity. So the present study was to synthesize and characterize a naphthalimide derivative 2-(2-benzylamine)-6-[(2-benzylamine)amino]-5-nitro-1H-benzo[de]-isoquinoline-1,3(2H)-dione (BBNID) and to evaluate the free radical activity.

EXPERIMENTAL

2,6-Diter-butyl-4-toluene (BHT) and 1,1-diphenyl-2-picrylhydrazyl radical (DPPH[•]) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Other chemicals were purchased from China National Medicine Group Shanghai Corporation (Shanghai, China). All chemicals and solvents used were of analytical grade. Infrared spectra were recorded on a PE Spectrum One FI-IR spectrometer as KBr pellets. ¹H NMR and ¹³C NMR spectra were recorded on a BRUKER AVANCE 500 spectrometer in CDCl₃. Mass spectra were recorded on BRUKER ESQUIRE HCT or VG ZAB-HS spectrometer. All elemental analyses were performed by PE 2400II analyzer. The data of single crystal were collected in a Rigaku Mercury CCD Area Detector.

Synthesis: 2-(2-Benzylamine)-6-[(2-benzylamine)amino]-5-nitro-1H-benzo[de]-isoquinoline-1,3(2H)-dione was synthesized as outlined in Fig. 1. 4-Bromo-1,8-naphthalic anhydride was treated with the mixture of potassium nitrate and sulphuric acid to offer 3-nitro-4-bromo-1,8-naphthalic anhydride in good yield¹⁰. The mixture of benzylamine (2.2 mmol), dimethylformamide (DMF, 10 mL) and 4-bromo-3-nitro-1,8-naphthalic anhydride (1 mmol) was refluxed at 100 °C for 6 h. Once cooled to room temperature, red crystals of BBNID were obtained in 88 % yields. BBNID was then

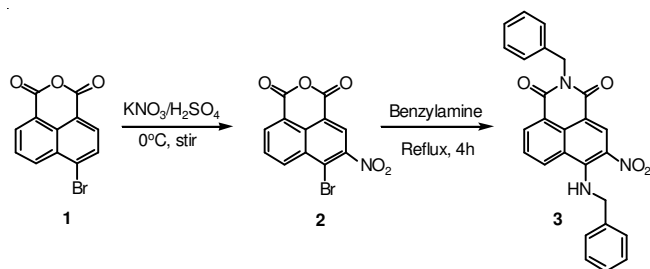


Fig. 1. Synthetic route of naphthalimides BBNID

characterized by FT-IR, NMR and elemental analysis: m.p. 233.5-234.7 °C (from DMF). IR (KBr, $\nu_{\max}$, cm^{-1}): 3441, 3060, 2923, 2851, 1699, 1655, 1601, 1537; ^1H NMR (500 MHz, CDCl_3) δ 10.02 (s, 1H, NH), 9.36 (s, 1H, H-Ar), 8.70 (d, $J = 7.4$ Hz, 1H, H-Ar), 8.63 (d, $J = 8.5$ Hz, 1H, H-Ar), 7.67 (d, $J = 8.3$ Hz, 1H, H-Ar), 7.26-7.65 (m, 10H, H-Ar), 5.38 (s, 2H, CH_2), 5.10 (s, 2H, CH_2); MS (ESI) m/z : 436 ($\text{M}^+ - 1$); Anal. Calcd. for $\text{C}_{26}\text{H}_{19}\text{N}_3\text{O}_4$: C, 71.39; H, 4.38; N, 9.61. Found: C, 71.25; H, 4.51; N, 9.72.

X-ray crystallography: An orange bar crystal of BBNID grown in DMF with dimensions of 0.25 mm $\times$ 0.20 mm $\times$ 0.04 mm was used for structural determination. Diffraction data were collected on a Rigaku Mercury CCD Area diffractometer by using graphite monochromated $\text{MoK}\alpha$ radiation ($\lambda = 0.71073$ Å). The structure was solved by direct methods with SHELXS-97 and refined on the F^2 by full-matrix least-squares method with SHELXL-97. All non-hydrogen atoms were refined anisotropically.

Scavenging activity: To evaluate the free radical scavenging activity, BBNID was allowed to react with a stable free radical, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH $^\bullet$) according to the literature¹¹ with a little modification. Briefly, each scavenger solution (0.1 mL) in DMF at different concentrations was added to solution [3.9 mL, 0.004 % (w/v)] of DPPH $^\bullet$ in ethanol. The reaction mixture was incubated at 37 °C. The scavenging activity on DPPH $^\bullet$ radical was determined by measuring the absorbance at 517 nm after 30 min. The scavenging activity was expressed as a percentage of scavenging activity on DPPH $^\bullet$ radical: $\text{SC \%} = [(A_{\text{control}} - A_{\text{test}}) / A_{\text{control}}] \times 100 \%$, where A_{control} is the absorbance of the control (DPPH $^\bullet$ solution without test sample) and A_{test} is the absorbance of the test sample (DPPH $^\bullet$ solution plus scavenger). The control contains all reagents except the scavenger.

RESULTS AND DISCUSSION

The crystal configuration of BBNID was confirmed by X-ray structural analysis. Experimental details for X-ray data collection were presented in Table-1 and the geometric parameters for BBNID were selected and listed in Table-2. The molecular structure of BBNID (3) is shown in Fig. 2. The molecular geometry is well comparable with that of 2-benzyl-6-(benzylamino)-1H-benzo[de]isoquinoline-1,3(2H)-dione¹², apart from the nitro group being substituted in the C-7 position. It features a six-membered imide ring in which two acyls are coordinated by N atom [$\text{C-N-C} = 124.2$ (5) Å]. All the atoms of 1,8-naphthalimide unit are almost coplanar. In the crystal packing of the compound 3 (Fig. 3), there are weak intermolecular C-H $\cdots$ O and N-H $\cdots$ O hydrogen bonds (Table-3), which makes the stabilization of the crystal structure.

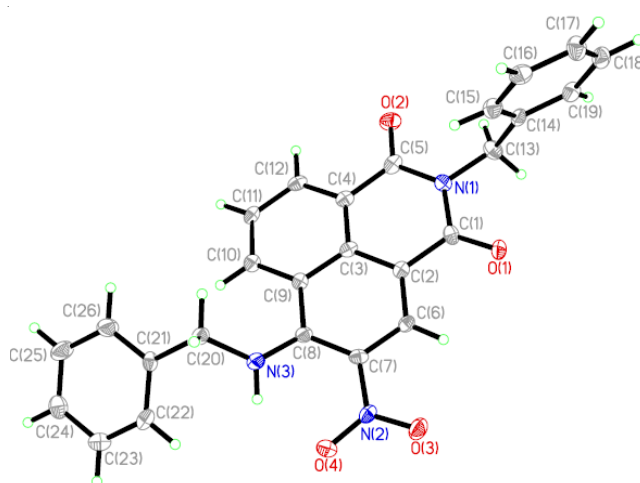


Fig. 2. General appearance of compound BBNID with the atoms represented by thermal vibration ellipsoids of 50 % probability

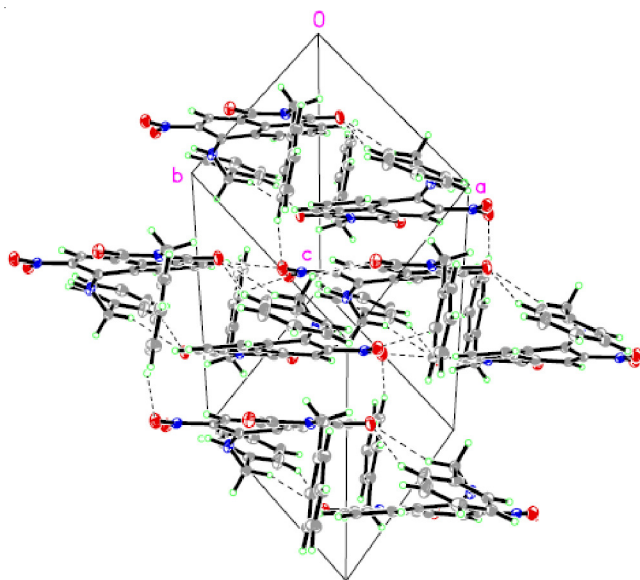


Fig. 3. Packing diagram for compound BBNID

TABLE-1
CRYSTALLOGRAPHIC DATA FOR COMPOUND BBNID

m.f.	$\text{C}_{26}\text{H}_{19}\text{N}_3\text{O}_4$
Formula weight	437.44
Crystal system, space group	Monoclinic, C2/c
Unit cell dimensions	$a = 8.8032$ (14) Å, $\alpha = 86.928$ (19)° $b = 8.822$ (2) Å, $\beta = 76.376$ (17)° $c = 13.994$ (4) Å, $\gamma = 72.284$ (16)°
Volume (Å 3)	1005.9 (4)
Crystal size (mm 3)	0.25 $\times$ 0.20 $\times$ 0.04
Temp. (K)	153 (2)
Z	2
D_{calc} (g cm $^{-3}$)	1.444
θ range for data collection	3.0 to 25.3°
$\mu_{\text{Absorp.}}$ (mm $^{-1}$)	0.10
Max. and min. transmission	0.996 and 0.756
Limiting indices	$-10 \leq h \leq 10$, $-10 \leq k \leq 10$, $-16 \leq l \leq 16$
$F(000)$	456
Reflections collected/unique	9964/3669 [$R(\text{int}) = 0.107$]
Data/restraint/parameters	3669/0/299
R [$I > 2\sigma(I)$]	$R_1 = 0.112$, $\omega R_2 = 0.267$
Goodness-of-fit on F^2	1.10
Largest diff. peak/hole (e/Å 3)	0.33 and -0.29

TABLE-2
SELECTED GEOMETRIC
PARAMETERS FOR COMPOUND BBNID

Bond	Dist. (Å)	Angle	Data (°)
O1–C1	1.223 (7)	C1–N1–C5	124.2 (5)
O2–C5	1.222 (7)	C1–N1–C13	116.5 (5)
O3–N2	1.231 (6)	C5–N1–C13	119.1 (5)
O4–N2	1.239 (6)	O3–N2–O4	121.0 (5)
N1–C1	1.393 (7)	O3–N2–C7	118.1 (5)
N1–C5	1.397 (7)	O4–N2–C7	120.8 (5)
N1–C13	1.483 (7)	C8–N3–C20	129.0 (5)
N2–C7	1.431 (7)	O1–C1–N1	119.8 (5)
N3–C8	1.362 (7)	O1–C1–C2	123.2 (5)
N3–C20	1.463 (7)	N1–C1–C2	117.0 (5)
C1–C2	1.473 (8)	C6–C2–C3	119.8 (5)
C2–C6	1.367 (8)	C6–C2–C1	119.4 (5)
C2–C3	1.411 (8)	C3–C2–C1	120.7 (5)
C3–C4	1.409 (8)	C4–C3–C2	120.0 (5)
C3–C9	1.425 (8)	C4–C3–C9	119.8 (5)

TABLE 3
HYDROGEN-BOND GEOMETRY FOR COMPOUND BBNID

D–H...A	D–H (Å)	H...A (Å)	D...A (Å)	D–H...A (°)
N3–H3...O4	0.88	1.99	2.615 (6)	127
C12–H12...O3 ⁱ	0.95	2.42	3.204 (7)	140
C15–H15...O4 ⁱⁱ	0.95	2.57	3.288 (7)	133
C16–H16...O3 ⁱⁱⁱ	0.95	2.51	3.389 (8)	153
C20–H20A...O2 ^{iv}	0.99	2.57	3.414 (7)	144
C20–H20B...O1 ⁱⁱ	0.99	2.47	3.239 (7)	134
C26–H26...O2 ^{iv}	0.95	2.48	3.335 (8)	150

Symmetry codes: (i) $x-1, y+1, z$; (ii) $-x+1, -y+1, -z+1$; (iii) $x-1, y, z$; (iv) $-x, -y+2, -z+1$

1,1-Diphenyl-2-picrylhydrazyl radical (DPPH[•]) scavenging activity evaluation is a rapid and convenient technique for screening the antioxidant activities of the antioxidants. Free radical scavenging activity of compound BBNID was measured against DPPH radical¹³. The values of IC₅₀, the effective concentration at which 50 % of the radicals were scavenged, were calculated to evaluate the free radical scavenging activity. A lower IC₅₀ value indicated greater antioxidant activity. IC₅₀ values of lower than 10 mg/mL usually implied

effective activities in antioxidant properties¹³. The IC₅₀ of commercial antioxidant BHT was also determined for comparison. The results showed that IC₅₀ of compound BBNID (which was equivalent to 0.02 mg/mL) was much lower than the standard value 10 mg/mL, indicating its good radical scavenging activity. It was important to note that BBNID displayed better free radical scavenging activity than the commercial antioxidant BHT (IC₅₀ = 65.85 µM), with IC₅₀ of 43.10 µM.

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