The Pharmaceutical and Chemical Journal, 2019, 6(2):91-102

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Synthesis and Biological Activity Studies of Substituted *N*-(1,3-Dioxohexahydro-2*H*-Isoindol-2-yl) Benzenesulfonamide Derivatives

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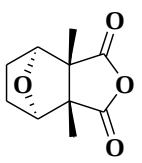
Abstract In this study, ten substituted *N* - ([1,3-dioxoindolin-2-yl] phenyl] sulfonyl) structures (compound **1-10**), which seven of them were original synthesized using conventional and microwave synthesis methods. In the first method the cis-1,2-cyclohexanecarboxylic anhydride and sulfa derivatives reacted under reflux for 2-3 hours in acetic acid. In the second method the cis-1,2-cyclohexanecarboxylic anhydride and sulfa derivatives were dissolved in DMF and radiated by microwave. Structure elucidation of the synthesized compounds were confirmed by UV, IR ^{1}H-NMR, ^{13}C-NMR with mass spectral methods and elemental analysis method. Anticancer activities of the compounds were studied by MTT assay. Synthesized compounds generally showed moderate or no cytotoxic activity against MCF7 (human breast cancer cell line). Among them, *N*-([1,3-Dioxoindolin-2-yl)phenyl]sulfonyl)6-amino-4,5-dimethoxypyrimidine (**7**), *N*-([1,3-Dioxoindolin-2-yl)phenyl]sulfonyl)-2-aminothiazole (**3**) and *N*-([1,3-Dioxoindolin-2-yl)phenyl]sulfonyl)aminopyrimidine (**10**) presented activity against MCF7 cancer cell lines with IC_{50} values of 71.5 ± 3.01μM, 87.9 ± 2.34and 89.3 ± 2.05 μM, respectively. Anti-inflammatory activity of the compounds were tested against LPS-induced nitrite production in RAW 264.7cell line. The compounds4-(1,3-dioxohexahydro-2H-isoindol-2-yl)-N-(5-methyl-1,2-oxazol-3-yl) benzenesulfonamide**(1),** 4-(1,3-dioxohexahydro-2H-isoindol-2-yl)-N-(6-methoxypyridazin-3-yl)benzenesulfonamide **(4)** and 4-(1,3-Dioxohexahydro-2H-isoindol-2-yl)benzenesulfonamide **(9)** showed nitrite production inhibitory activity 24.43 ± 3.16, 9.73 ± 1.04, and 6.44 ± 2.48 μM, respectively. Compound 4 exhibited the highest anti-inflammatory activity by suppressing the NO production. These compounds might be candidate to inflammatory therapy for further development.

Keywords Anticancer activity, Anti-inflammatory activity, Breast cancer MCF-7, Pharmaceutical analysis

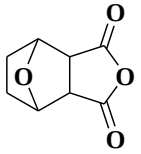
1. Introduction

Today, cancer and inflammatory diseases are the most important health problems around the world. The functional relationship between inflammation and cancer is not new. In 1863, Virchow hypothesized that the origin of cancer was sites of chronic inflammation [1]. Today, the relationship between inflammation-immunity and cancer is more widely accepted. Several excellent reviews are found concerning the acquired immune response to cancer, which is related to the inflammatory response [2-3]. This evidence has encouraged scientists to design new anti-inflammatory drugs that may be useful for cancer treatment.

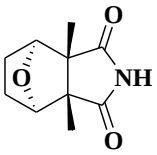
Mylabris is the dried body of the blister beetle and has been used in Chinese medicine for thousands of years for the treatment of malignant tumors, such as breast, colorectal, hepatoma cancer, and abdominal malignancies [4]. To date, various anti-cancer drugs have been isolated from Mylabris; e.g., cantharidin (CTD), norcantharidin (NCTD), cantharimide and norcantharimide.



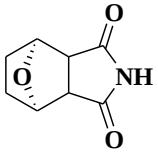
Cantharidin (CTD)



Norcantharidin (NCTD)

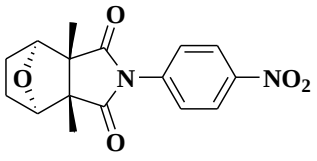


Cantharimide

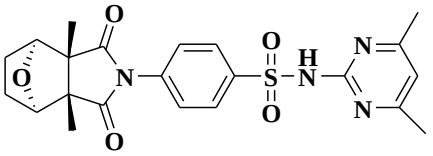


Norcantharimide

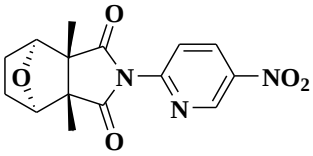
Many cantharimide and norcantharimide analogs (I-III) bearing several different substituent's at *N*-position were synthetized and screened for their anticancer activity [4-7].



I

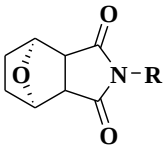


II

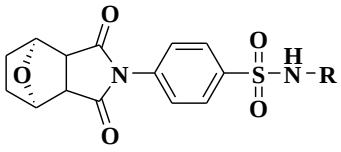


III

As shown in the examples given immediately below, a series of norcantharimide and phthalimide analogs bearing hybrid structures were reported to have enhanced bioavailability and transportability through the cell membrane compared to the norcantharimide and phthalimide [8].

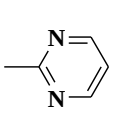


IV-X



XI-XVIII

R; **H** ,



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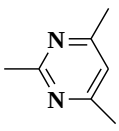

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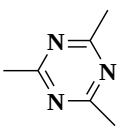

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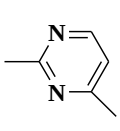

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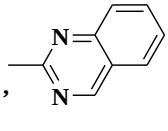

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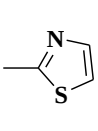

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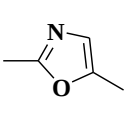
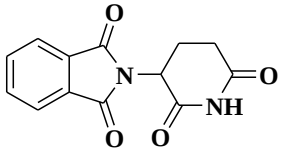


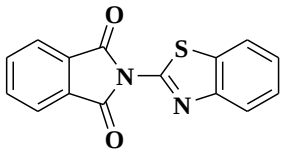
Sulfonamides are interesting aryl amines showing antibacterial, carbonic anhydrase inhibitory, hypoglycemic and antithyroid activities [9-14]. The analogs of norcantharimide and phthalimides with sulfonamides were also prepared and found to promote anti-inflammatory and anticancer activity (IV-XVIII) [15-16].

Phthalic anhydride and phthalimide are the aromatic derivatives of cyclohexanedicarboxylic anhydride/imide. The most important derivative is thalidomide, which has antitumor, anti-inflammatory, antimicrobial and immunomodulatory activities [17-20]. Recently, it was reported that *N*-substituted cyclic imide derivatives possess inflammatory activity on the inhibition of tumor necrosis factor-α (TNF-α) (IXX-XX) [15-21]. LASSBio468 was

found to have a sulfonyl-thiomorpholine moiety that showed potent inhibitory activity on LPS-induced neutrophil recruitment at ED_{50}=2.5 mg/kg, correlated with its inhibitory effect at the TNF-α level [16].

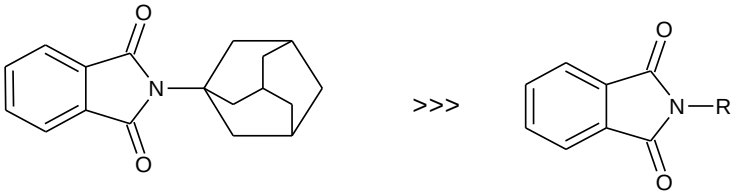


Thalidomide



LASSBio468

N-alkylated phthalimide analogs bearing adamantyl and several R groups at the *N*-position show very potent bi-directional TNF-α production-regulating activity. Among these series, 4-pentylphenyl-, 1-adamantyl- and 2,4-dimethylphenyl-substituted compounds exhibit the best activity [15].



R = 3-penthyl > R = cyclohexyl > R = t-butyl > R = n-butyl

In the light of previous research, we aimed to synthesize a series of *N*-(1,3-dioxohexahydro-2*H*-isoindol-2-yl)benzenesulfonamide derivatives. They were planned as N-substituted phthalimide derivate, structurally designed as a hybrid of thalidomide and aryl sulfonamides, which were expected to show anticancer and anti-inflammatory activities.

2. Material and Methods

2.1. Chemistry

All materials were commercially available and used without further purification. The melting points of the compounds were determined in Celsius (°C) using a Mettler Toledo FP 900 Thermo System Digital melting point apparatus, and the values are uncorrected. Microwave irradiation was carried out with a microwave reactor (MicroSYNTH, Milestone, Italy). UV spectra were recorded at a concentration of 2×10^{-5} M in methanol with a quartz cell of 1-cm path length by a UV-VIS Agilent 8453 spectrometer. Infrared spectra were recorded on a Perkin-Elmer Spectrum One series FT-IR apparatus (Version 5.0.1), and using potassium bromide pellets, the frequencies were expressed in cm^{-1}. The ^{1}H-NMR and ^{13}C –NMR were recorded in deuterated-dimethyl sulfoxide (DMSO-d_6) with a Varian Mercury-400 FT-NMR spectrometer (Varian Inc., Palo Alto, CA, USA) using tetramethylsilane (TMS) as the internal reference. The chemical shifts were reported in parts per million (ppm). The M+1 peaks were determined by the Shimadzu LC/MS ITTOF system (Shimadzu, Tokyo, Japan). The elemental analysis was performed on a LECO932CHNS (Leco-932, St. Joseph, MI, USA) instrument.

2.1.1. Procedure A: Conventional synthesis of Compounds 1-10

The compounds were prepared by stirring 0.0013 mol (0.20 g) of *cis*-1,2-cyclohexane carboxylic anhydride and 0.0013 mol of corresponding sulfa drugs in 10 ml of acetic acid under reflux for two to three hours. Then, 20 ml of distillated water was added to the solution at room temperature and filtered. The obtained crude products were crystallized in ethanol, and then filtered and dried in vacuum.

2.1.2. Procedure B: Microwave-assisted synthesis of Compounds1-10

The compounds were prepared by stirring 0.0013 mol (0.20 g) of c*is*-1,2-cyclohexane carboxylic anhydride and 0.0013 mol of sulfa drugs in 0.4 ml of dimethylformamide at room temperature until they dissolved. Then, the mixture was subjected to a power of 200 Watt in a MicroSYNTH Microwave reactor for four minutes at 90 °C. The

mixture was then cooled, and 20 ml of distillated water was added. The obtained crude products were crystallized in ethanol, and then filtered and dried in vacuum (See table 1).

Table 1: Microwave conditions

Step	Time (min)	Power (W)	Temp (°C)
1	5	250	90
2	4	200	90

4-(1,3-dioxohexahydro-2H-isoindol-2-yl)-N-(5-methyl-1,2-oxazol-3-yl)benzenesulfonamide (1)

0.0013 mol of *cis*-1,2-cyclohexane carboxylic anhydride (0.20 gr) and 0.0013 mol of sulfamethoxazole (0.33 gr) were reacted as described in the procedures A and B. M.p.: 213 °C. UV (MeOH, λ_{max}, nm): 240 (log ε: 6.08), 395 (log ε: 8.30). FT-IR (KBr, ν_{max}, cm^{-1}): 3475 (N-H), 3075 (C-H, aromatic), 2934 (aliphatic C-H), 1702 (O=C-N-C=O), 1170 (SO_2-NH). ^{1}H-NMR (400 MHz) (DMSO-d^6/TMS, δ, ppm): 11.65 (s, 1H, SO_2-N**H**), 7.60-8.00 (m, 4H, Ar), 6.20 (s, 1H, H-4 oxa), 3.10 (m, 2H, C**H**), 2.30 (s, 3H, **CH_3**), 1.80 (m, 4H, C**H_2**), 1,33 (m, 4H, C**H_2**). LC-MS (m/z): 390.43(M^+) ($C_{18}H_{19}N_3O_5S$), 254 $(C_{10}H_{12}N_3O_3S)^+$. Calcd for $C_{17}H_{17}N_3O_6S$: C, 52; H, 4.34; N, 10.74. Found: C, 51.68; H, 4.04; N, 10.34.

N-[4-(1,3-diioxohexahydro-2H-isoindol-2-yl)benzene-1-sulfonyl]acetamide (2)

0.0013 mol of *cis*-1,2-cyclohexane carboxylic anhydride (0.20 gr) and 0.0013 mol of sulfacetamide (0.28 gr) were reacted as described in the procedures A and B. M.p.: 178 °C. UV (MeOH, λ_{max}, nm): 207 (log ε: 8.01), 239 (log ε: 8.08), 398 (log ε: 8.30). FT-IR (KBr, ν_{max}, cm^{-1}): 3273 (N-H), 300 (C-H, aromatic), 2942 (C-H, aliphatic), 1703 (O=C-N-C=O), 1597 (HN-C=O), 1336 (SO_2NH). ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 12.76 (s, 1H, SO_2-N**H**), 7.40-8.00 (m, 4H, Ar), 3.05 (m, 2H, **C**H), 2.00 (s, 3H, -COC**H_3**), 1.80 (m, 4H, C**H_2**), 1.40 (m, 4H, C**H_2**). ^{13}C-NMR (400 MHz) (DMSO-d6/TMS, δ, ppm): 178.13, 168.91, 138.51, 136.82, 128.30, 127.31, 39.42, 23.28, 23.23, 21.45. LC-MS (m/z): 351.39 $C_{16}H_{18}N_2O_5S$ (M^+). Calcd for $C_{16}H_{18}N_2O_5S$: C, 54.86; H, 5.14; N, 8.00. Found: C, 55.00; H, 4.68; N, 8.34.

***4*-(1,3-dioxohexahydro-2H-isoindol-2-yl)-N-(1,3-thiazol-2-yl)benzenesulfonamide (3)**

(CAS Registry Number: 1802658-09-8)

4-(1,3-dioxohexahydro-2H-isoindol-2-yl)-N-(6-methoxypyridazin-3-yl)benzenesulfonamide (4)

0.0013 mol of *cis*-1,2-cyclohexane carboxylic anhydride (0.20 gr) and 0.0013 mol of sulfamethoxypyridazine (0.36 gr), were reacted as described in the procedures A and B. The compound was crystallized from ethanol. M.p.: 240 °C . UV (MeOH, λ_{max}, nm): 204 (log ε: 8.01), 222 (log ε: 8.05), 325 (log ε: 8.21). FT-IR (KBr, ν_{max}, cm^{-1}):3466 (N-H), 3080 (C-H, aromatic), 2942 (C-H), 1702 (O=C-N-C=O), 1168 (SO_2NH), 2855 (OCH_3). ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 7.90 (m, 4H, Ar) 7.40 (m, 2H, Ar), 3.8 (s, 3H, -O-C**H_3**), 3.10 (m, 2H, C**H**), 1.77 (m, 4H, C**H_2**) 1.33 (m, 4H, C**H_2**).

^{13}C-NMR (400 MHz) (DMSO-d6/TMS, δ, ppm): 178.25, 135.31, 127.33, 126.72, 54.55, 39.50, 23.28, 21.43. LC-MS (m/z): 417.45 ($C_{11}H_{13}N_3O_3S$) (M^+)), 267.30 (C_{19} H_{20} $N_4O_5S)^+$. Calcd for $C_{11}H_{13}N_3O_3S$: C, 55.00; H, 4.80; N, 13.4. Found: C, 55.20; H, 4.40; N, 12.95.

N-[4-(1,3-dioxohexahydro-2H-isoindol-2-yl)benzene-1-sulfonyl]benzamide (5)

0.0013 mol of *cis*-1,2-cyclohexane carboxylic anhydride (0.20 gr) and 0.0013 mol of sulfabenzamide (0.36 gr) were reacted as described in the procedures A and B. M.p.: 245 °C. UV (MeOH, λ_{max}, nm): 206 (log ε: 8.01), 238 (log ε: 8.07), 393 (log ε: 8.29).FT-IR (KBr, ν_{max}, cm^{-1}): 3296 (C-H, aromatic), 3218 (N-H), 3064 (C-H, aromatic), 2954 (aliphatic, C-H), 1781 (O=C-N-C=O), 1694 (C=O, amides),1245 (SO_2NH). ^{1}H-NMR (400 MHz) (DMSO-d6/TMS, δ, ppm): 12.60 (s, 1H, SO_2-N**H**), 8.10-7.40 (m, 9H, Ar), 3.10 (m, 2H, C**H**),1.90 (m, 4H, C**H_2**), 1.40 (m, 4H, C**H_2**).^{13}C-NMR (400 MHz) (DMSO-d6/TMS, δ, ppm): 178.12, 165.58, 138.62, 136.83, 133.36, 131.34, 128.61, 128.47, 128.45, 127.29, 39.50, 23.27, 21.44.LC-MS (m/z): 413.45 (M^+) ($C_{21}H_{20}N_2O_5S$), 183.18 ($C_7H_5NO_3S$), 267.34 Calcd for $C_{13}H_{17}NO_3S$: C, 61.10; H, 4.90 ; N, 6.8. Found: C, 60.60; H, 4.40; N, 7.10.

4-(1,3-dioxohexahydro-2H-isoindol-2-yl)-N-(4-methylpyrimidin-2-yl)benzenesulfonamide (6)

(CAS Registry Number: 309267-54-7)

4-(1,3-dioxohexahydro-2H-isoindol-2-yl)-N-(4,5-dimethoxypyrimidin-2-yl)benzenesulfonamide (7)

0.0013 mol of *cis*-1,2-cyclohexane carboxylic anhydride (0.2 gr) and 0.0013 mol of sulfadoxine (0.40 gr) were reacted as described in the procedures A and B. M.p.: 190°C . UV (MeOH, λ_{max}, nm): 240 (log ε: 8.08), 261 (log ε: 8.12). FT-IR (KBr, ν_{max}, cm^{-1}): 3260 (N-H), 3078 (C-H, aromatic), 2936 (C-H, aliphatic), 1783 (O=C-N-C=O), 1373 (OCH_3), 1305(OCH_3), 1258 (SO_2NH). ^{1}H-NMR (400 MHz) (DMSO-*d*6/TMS, δ, ppm): 11.27 (s, 1H, SO_2-N**H**), 8.10 (m, 1H, Ar), 7.50 (m, 4H, Ar), 3.90 (s, 3H, -O-C**H$_3$**), 3.70 (s, 3H, -O-C**H$_3$**), 3.31 (m, 2H, C**H**), 1.80 (m, 4H, C**H$_2$**), 1.40 (s, 2H, C**H$_2$**).^{13}C-NMR (400 MHz) (DMSO-d6/TMS, δ, ppm): 178.64, 162.18, 156.38, 151.98, 150.71, 149.34,140.64, 136.59, 128.54, 127.97, 127.60, 125.76, 60.68, 54.56, 39.55, 23.73, 21.90.LC-MS (m/z): 447.48 (M^+) ($C_{20}H_{22}N_4O_6S$), 311.08 ($C_{12}H_{15}N_4O_4S)^+$. : Cald. for $C_{20}H_{22}N_4O_6S$: C, 57.00; H, 5.00; N, 14.00. Found: C, 56.75; H, 5.15; N, 14.30.

4-(1,3-Dioxohexahydro-2H-2-yl)-N-(pyrimidin-2-yl)benzenesulfonamide (8)

(CAS Registry Number: 431918-18-2)

4-(1,3-Dioxohexahydro-2H-isoindol-2-yl)benzenesulfonamide (9)

0.0013 mol of *cis*-1,2-cyclohexane carboxylic anhydride (0.2gr) and 0.0013 mol of sulfanilamide (0.22 gr), 10 ml of acetic acid were reacted as described in the procedures A and B. M.p.: 256 °C.UV (MeOH, λ_{max}, nm): 205 (log ε: 8.01), 234 (log ε: 7.77). FT-IR (KBr, ν_{max}, cm^{-1}):3258 (N-H), 3096 (C-H, aromatic), 2928 (C-H, aliphatic), 1783 (O=C-N-C=O), 1167 (SO_2NH). ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 7.90-7.50 (m, 4H, Ar), 3.50 (s, 2H, N**H$_2$**), 3.30 (m, 2H, C**H**), 1.90 (m, 4H, C**H$_2$**), 1.40 (m, 4H, C**H$_2$**). ^{13}C-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 178.26, 143.52, 135.28, 127.40, 126.35, 39.50, 23.28, 21.40. LC-MS (m/z): ($C_{14}H_{16}N_2O_4S$); 309.35 (M^+) $(C_{14}H_{16}N_2O_4S)^+$.Calcd. for $C_{14}H_{16}N_2O_4S$; C, 54.50; H, 5.20; N, 9.10. Found: C, 54.90; H, 5.15; N, 9.40.

4-(1,3-Dioxohexahydro-2H-2-yl)-N-(4,6-dimethylpyrimidin-2-yl)benzene1sulfonamide (10)

0.0013 mol of *cis*-1,2-cyclohexane carboxylic anhydride (0.2gr) and 0.0013 mol of sulfamethazine (0.22 gr) were reacted as described in the procedures A and B. The compound was crystallized from ethanol. This compound is soluble in acetone, hot ethanol, methanol and DMSO, but insoluble in water. M.p.: 213 °C. UV (MeOH, λ_{max}, nm): 311 (log ε: 8.19). FT-IR (KBr, ν_{max}, cm^{-1}): 3464 (N-H), 3056 (C-H, aromatic), 2941 (C-H, aliphatic), 1781 (O=C-N-C=O), 1163 (SO_2NH). ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 12.0 (s, 1H, SO_2-N**H**), 7.70-8.20 (m, 4H, Ar), 6.80 (d, 1H, pyr.), 3.05 (m, 2H, C**H**), 2.20 (s, 6H, C**H$_3$**), 1.90 (m, 4H, C**H$_2$**), 1.60 (m, 4H, C**H$_2$**).

^{13}C-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 178.22, 155.94, 135.65, 128.57, 126.66, 39.50, 23.27, 22.64, 21.43. LC-MS (m/z): ($C_{18}H_{24}N_4O_4S$) 415.49 (M^+), 267.34 $(C_{12}H_{17}N_3O_2S)^+$. Calcd. for $C_{18}H_{24}N_4O_4S$: C, 56.00; H, 4.70; N, 14.50. Found: C, 55.90; H, 5.15; N, 14.10

2.2. Pharmacological activities

2.2.1. Cell Culture Reagents and Culture Conditions

The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, UK), supplemented with 10% fetal bovine serum (FBS) (Gibco, USA), and 1% penicillin and streptomycin (Gibco, USA). Each cell line was maintained in an incubator at 37 °C supplied with 5% CO_2 and 95% air.

2.2.2. Anticancer activity

The anticancer activity of the compounds was examined on the human breast adenocarcinoma cell line (MCF7) by MTT assay. In previous studies, compounds **3**, **6** and **8** were screened for *in vitro* anticancer activity against five human cancer cell lines; T47D, NCI H-522, HCT-15, PA-1, and Hep G2.

The cytotoxic activities of the compounds were investigated on the MCF7 human breast adenocarcinoma cell line (ATCC HTB-22) by MTT assays [23]. Serial dilutions from 10^{-4} to 10^{-7} M were used, and 5-FU (Roche, Germany) was the reference compound for anticancer activity.

The experiments were carried out following the instructions given in "Cell Proliferation Kit I (MTT)" (Roche, Catalog Number: 11 465 007 001). The cancer cells were seeded into 96-well plates and allowed to adhere for 24 h before the drugs were introduced. Following this step, each medium in the wells was replaced with fresh medium and different concentrations (10^{-4} - 10^{-7} M) of the compounds were added and incubated for 24 h. After a 48h

incubation period, each well was treated with 10 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, final concentration 0.5 mg/mL) in the culture medium. Following a 4h incubation period to allow the metabolism of MTT by mitochondrial dehydrogenases of viable cells to form an insoluble formazan product, 100 µL of a solution containing 10% SDS in 0.01M HCl was added into each well. The plates were shaken to maximize the solubilization of the formazan crystals and they were read at 550-600 nm using a scanning multi-well spectrophotometer (SpectraMax i3, Molecular Devices). Each set of experiments was performed in triplicate in three independent assays.

2.2.3. Anti-inflammatory Activity

The anti-inflammatory activities of the compounds were examined by measuring nitrite concentrations using a colorimetric method based on the Griess reaction on RAW 264.7 macrophage cells provided by Yeditepe University, Faculty of Engineering, Department of Genetics and Bioengineering (Istanbul, Turkey).

Nitrite Assay: The nitrite inhibition activity of the tested compounds was evaluated by measuring the nitrite concentrations using a colorimetric method based on the Griess reaction. RAW 264.7 cells were seeded into a 48-well culture plate at the density of 1 x 105 cells per well and incubated for 24 h. The cells were then pretreated with the compounds and the reference molecule, acetylsalicylic acid (500 µM). Two hours later, the cells were stimulated with LPS (1µg/ml), and after 22 h, the nitrite concentration in the medium was measured by adding 50 µl Griess reagent [1% sulfanilamide (Sigma-Aldrich, USA) and 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride (Sigma-Aldrich, USA) in 5 % phosphoric acid (Mettler, Switzerland)] to 50 µl of medium for 10 min. The absorbance was measured at 570 nm using a microplate reader (Microplate photometer, Multiskan Ascent, Finland). A sodium nitrite (Fluka Chemika, Germany) standard curve was used to calculate the amount of nitrite in the test samples.

2.2.4. Statistics

All results were expressed as the mean ± SD of experiments. Statistical significance was determined by one-way ANOVA followed by Turkey's test using a computerized statistical program. The data were considered statistically significant if $p<0.05$.

3. Results and discussion

3.1. Chemistry

The synthetic routes for substituted N-(1,3-dioxohexahydro-2*H*-isoindol-2-yl) benzenesulfonamide compound (**1-10**) are summarized in Figure 1.

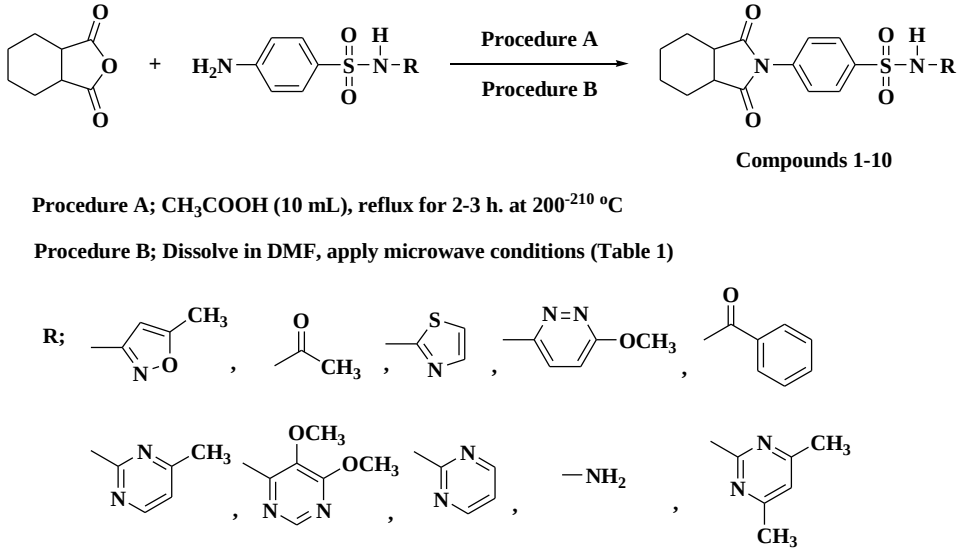
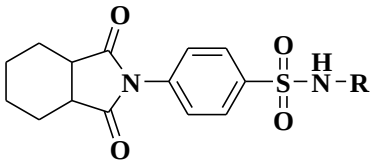


*Figure 1: The general synthetic pathway of target compounds **1-10**.*

Two procedures were applied for the synthesis of the target compounds. First, the compounds were prepared by the reaction of *cis*-1,2-cyclohexane carboxylic anhydride with the corresponding sulfa derivatives in acetic acid under reflux for two to three hours. Second, the *cis*-1,2-cyclohexanecarboxylic anhydride and sulfa derivatives were dissolved in DMF, and then irradiated using a microwave reactor under the conditions given in Figure 1. The yield of the compounds was between 30% - 100% in both methods (See table 2).

Table 2: Structure, % yields and melting point of the synthesized compounds



COMPOUND		% YIELD		M.P. ^{0}C	
No	**R**	**RFLX**	**MW**	**RFLX**	**MW**
1		31	97	213	213
2		98	98	178	178
3		100	97	230	230
4		60	100	240	240
5		100	97	245	245
6		100	100	318	318
7		100	99	190	190
8		90	100	254	254
9		40	95	256	256
10		90	90	213	213

The structure and purity of the compounds were examined by UV, IR, ^{1}H-NMR, ^{13}C-NMR, mass spectra and elemental analyses. The yields of the compounds were higherthan the values reported in the literature [22].

3.2. Anticancer activity

Among these, compound **3** exhibited anticancer activity against NCI H-522 and Hep G2 with the IC_{50} values 30 µM and 36 µM, respectively. Compounds **6** and **8** were not active. The assay was performed in two steps. First, compounds 1-10 were tested at 10^{-4} M concentration. In the second step, 10^{-5} M - 10^{-7} M concentrations of the selected compounds that indicated more than 50% inhibitory activity at initial concentrations were analyzed. Compounds **3**, **7** and **10** were found to possess anticancer activity with the IC_{50} values 87.9 ± 2.34, 71.5 ± 3.01, and 89.3 ± 2.05 µM, respectively. Statistical comparisons were undertaken using one-way analysis of variance (ANOVA) and the module of GraphPad Prism software Version 7.02 (La Jolla, California, USA). The differences in the mean values were considered significant when $P<0.05$. According to the results, it can be clearly stated that compound **3** had anticancer effect on liver, lung and breast adenocarcinoma cell lines.

The cytotoxic activity results of the synthesized compounds and Endpoint cell index values of the compounds are given in Table 3 and Figure 1-5 respectively.

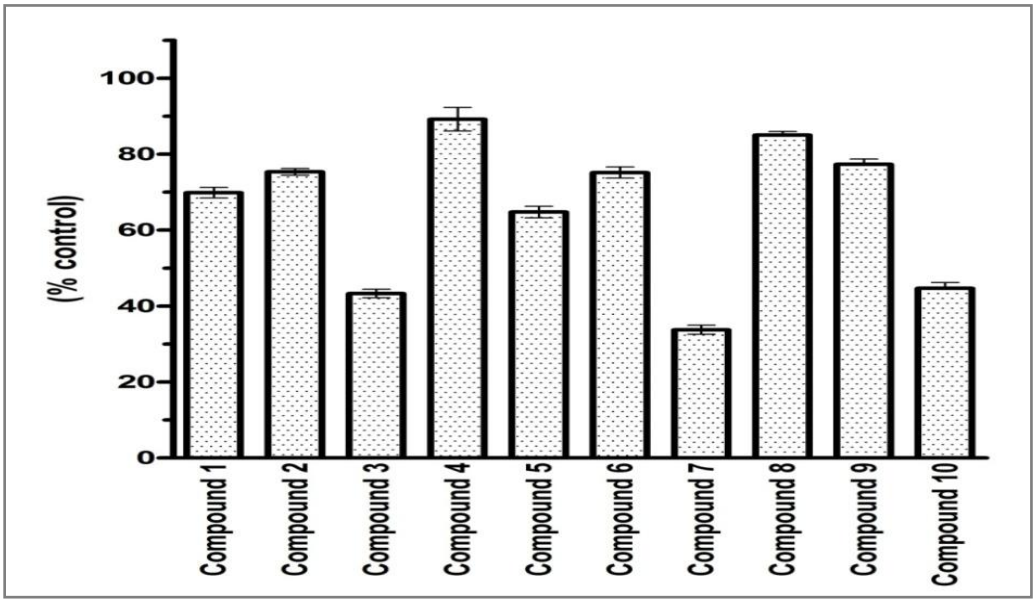


Figure 2: Endpoint cell index values (24 h incubation with compounds)

*Figure 2: The effect of compounds 1–10 at the concentration of 10^{-4}M on the viability of MCF7 (*P< 0.05, ***P< 0.001 determined by one-way ANOVA using Dunnet's multiple comparison test)*

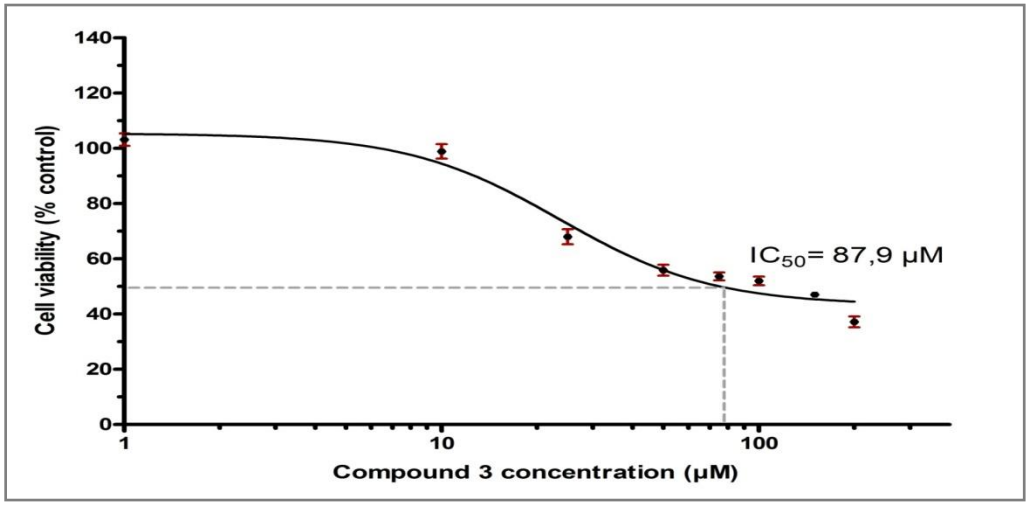


Figure 3: Endpoint cell index values (24 h incubation with compound 3)

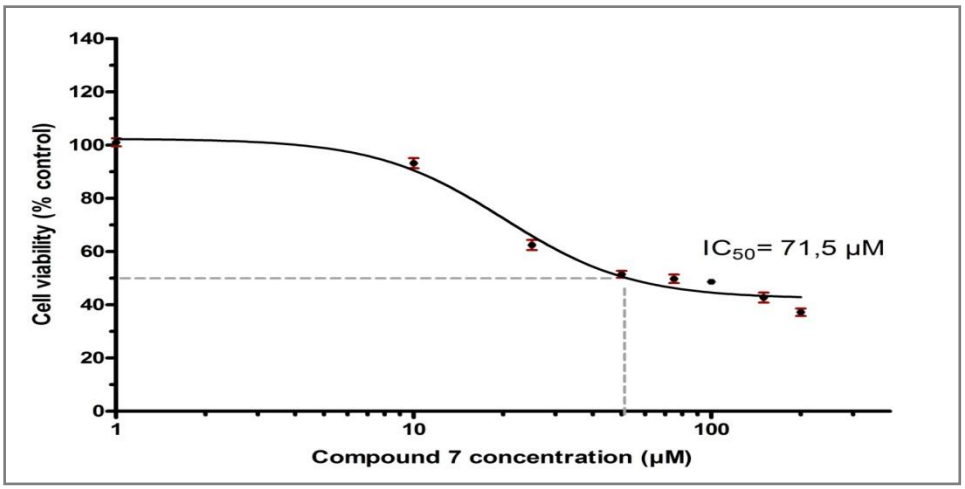


Figure 4: Endpoint cell index values (24 h incubation with compound 7)

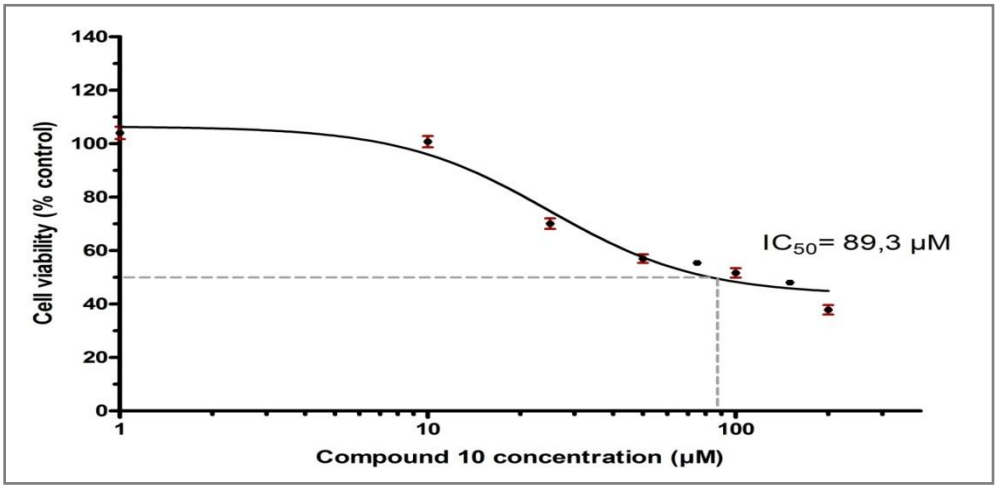


Figure 5: Endpoint cell index values (24 h incubation with compound 10)

Table 3: The IC_{50} values of the synthesized compounds 1-10and the reference molecule 5-FU against human breast cancer cell line (MCF7) by MTT assay.

Compound	**R**	**% cell viability MCF7 at 100 µM**	**IC_{50} µM**
1	5-methyl-1,2-oxazol-3-yl	69.84 ± 1.37	NA
2	acetyl	75.35 ± 1.21	NA
3	1,3-thiazol-2-yl	43.31 ± 1.46	87.9 ± 2.34
4	1,2-diazine-6-methoxy-3-yl	89.19 ± 1.54	NA
5	phenylcarbonyl	64.75 ± 3.08	NA
6	1,3-diazine-6-methyl-2-yl	75.16 ± 1.13	NA
7	1,3-diazine-5,6-dimethoxy-4yl	33.75 ± 0.88	71.5 ± 3.01
8	1,3-diazine-2-yl	85.01 ± 0.96	NA
9	H	77.35 ± 1.35	NA
10	1,3-diazine-4,6-dimethyl-2-yl	44.68 ± 1.48	89.3 ± 2.05
5-FU	5-fluorouracil	27.38 ± 0.44	3.5 ± 0.97

NA: Non-active

Figure 3. The effects of active compounds on cell viability. **a** The dose-response curve of compound 3 for cell viability (R^2 = 0.92). For the MCF7 cells, the IC_{50} value of compound 3 was 87.9 μM. The graph [sigmoidal dose-response (variable slope) curve fit] presents the mean ± SD of three independent experiments analyzed together (n = 9).**b** The dose-response curve of compound 5 for cell viability (R^2 = 0.90). For the MCF7 cells, the IC_{50} value of compound 7 was 71.5 μM. The graph [sigmoidal dose-response (variable slope) curve fit] presents the mean ± SD of three independent experiments analyzed together (n = 9). **c** The dose-response curve of compound 10 for cell viability (R^2 = 0.97). For the MCF7 cells, the IC50 value of compound 3 was 89.3 μM. The graph [sigmoidal dose-response (variable slope) curve fit] presents the mean ± SD of three independent experiments analyzed together (n = 9).

3.3. Anti-inflammatory Activity

In this study, the synthesized compounds were tested for their inhibitory activity against LPS-induced nitrite production in RAW 264.7 cells, and the results are summarized in Table 4. Among the tested compounds, **1**, **4** and **9** showed nitrite production inhibitory activity while compound 4 exhibited the highest anti-inflammatory activity by suppressing the NO production. The compounds were also analyzed for their cytotoxicity against RAW 264.7 macrophages using the MTT assay. No significant cytotoxic activities were observed at any of the tested concentrations. MCF7 cancer cell lines with IC_{50} values of 71.5 ± 3.01μM, 87.9 ± 2.34and 89.3 ± 2.05 μM, respectively. Anti-inflammatory activity of the compounds were tested against LPS-induced nitrite production in RAW 264.7cell.Among the tested compounds4-(1,3-dioxohexahydro-2H-isoindol-2-yl)-N-(5-methyl-1,2-oxazol-3-yl) benzenesulfonamide **(1),** 4-(1,3-dioxohexahydro-2H-isoindol-2-yl)-N-(6-methoxypyridazin-3-yl)benzenesulfon amide **(4)** and 4-(1,3-Dioxohexahydro-2H-isoindol-2-yl)benzenesulfonamide **(9)** showed nitrite production inhibitory activity 24.43 ± 3.16, 9.73 ± 1.04, and 6.44 ± 2.48 μM, respectively.

Table 4: The inhibitory effect of compounds **1-10** and the reference molecule ASA ((500μM) on the nitric oxide (NO) levels in the LPS-stimulated macrophage cells

Compound	R	Cell Viability	NO Inhibition
1	5-methyl-1,2-oxazol-3-yl	90.63 ± 7.77	9.73 ± 1.04
2	acetyl	89.44 ± 6.18	ND
3	1,3-thiazol-2-yl	93.00 ± 4.38	ND
4	1,2-diazine-6-methoxy-3-yl	88.52 ± 4.15	24.43 ± 3.16
5	phenylcarbonyl	96.03 ± 4.07	ND
6	1,3-diazine-6-methyl-2-yl	87.30 ± 8.39	ND
7	1,3-diazine-5,6-dimethoxy-4-yl	91.22 ± 3.00	ND
8	1,3-diazine-2-yl	93.10 ± 4.69	ND
9	H	87.82 ± 6.71	6.44 ± 2.48
10	1,3-diazine-4,6-dimethyl-2-yl	90.23 ± 6.31	ND
ASA	ASA	100.22 ± 10.07	40.89 ± 3.36

ASA: Acetylsalicylic acid, ND: Non-detectable, *: $P<0.05$

4. Conclusion

In conclusion, A series of *N*-(1,3-Dioxohexahydro-2*H*-isoindol-2-yl)benzenesulfonamide (compounds 1-10), were synthesized using cis-1,2-cyclohexanecarboxylic anhydride and sulfa derivatives in acetic acid under reflux or dissolving the *cis*-1,2-cyclohexanecarboxylic anhydride and sulfa derivatives in DMF and subsequent irradiation in a microwave reactor. All synthesized compounds were investigated for anticancer and anti-inflammatory properties. To the best of our knowledge, no research has been undertaken to investigate the anti-inflammatory activity of *N*-(1,3-dioxohexahydro-2*H*-isoindol-2-yl)benzenesulfonamide derivatives. Among the tested compounds, compounds **3**, **7** and **10** were found to possess anticancer activity with the IC_{50} values 87.9 ± 2.34, 71.5 ± 3.01, and 89.3 ± 2.05 μM, respectively while compounds **1**, **4** and **9** showed nitrite production inhibitory activity while compound **4** exhibited the highest anti-inflammatory activity by suppressing the NO production. These compounds might be candidate to cancer and inflammatory therapy for further development.

Conflict of Interest

The authors confirm that they do not have any conflict of interest concerning the content of this article.

Acknowledgements

The authors are grateful to Yeditepe University for allowing the use of facilities to Prof. Dr. Hakan Göker for performing the instrumental analysis

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