

Synthesis and *In Vitro* Cytotoxicity of 1,3-Dioxoindan-2-Carboxylic Acid Arylamides

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A series of 1,3-dioxoindan-2-carboxylic acid arylamides were synthesized and evaluated for *in vitro* cytotoxicity against four human cancer cell lines (HOP62, SK-OV-3, MD-MB-468 and T-47D). The most active was compound **3e** (1.2 μ M against SK-OV-3 cell line) bearing a 4-methyl substituent.

Key words: Antitumor agents, Cytotoxic activity, TAS-103

INTRODUCTION

The development of drug resistance is one of the causes of the severe limitation to chemotherapy in cancer patients. The use of inhibitors of topoisomerase I and II has shown a synergistic effect against resistant tumor cells (Cortes and Pinero, 1994; Bonner and Kozelsky, 1996). Recently, interest has focused on the mixed topoisomerase I/II inhibitors as they are more effective at overcoming "atypical" drug resistance. Examples of such mixed inhibitors, which show broad-spectrum activity against solid tumors and are in clinical trials, include *N*-[2-(dimethylamino)-ethyl]acridine-4-carboxamide (DACA) (**1**) (Atwell *et al.*, 1987; Baguley *et al.*, 1995; Dittrich *et al.*, 2003) and 6-[[2-(dimethylamino)-ethyl]amino]-3-hydroxy-7*H*-indeno[2,1-*c*]quinolin-7-one dihydrochloride (TAS-103) (**2**) (Aoyagi *et al.*, 1999; Minderman *et al.*, 2000) (Fig. 1). DACA is a tricyclic carboxamide-based cytotoxic agent that binds to DNA by intercalation and stimulates DNA cleavage by inhibition of both topoisomerases I and II. TAS-103 is a tetracyclic indenoquinoline-based cytotoxic agent that binds to DNA.

The synthesis and *in vitro* cytotoxicities of 2-substituted 4-methylquinoline derivatives (Lee *et al.*, 2000) and cyclopentaquinoline derivatives (Lee *et al.*, 2001) have been previously reported. These were designed as truncate

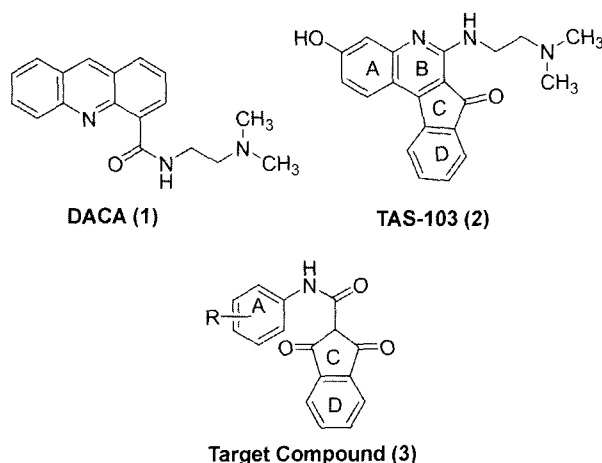


Fig. 1. Chemical structures of DACA (**1**), TAS-103 (**2**), and 1,3-dioxoindan-2-carboxylic acid arylamides (**3**)

analogues of TAS-103. Our previous results revealed that both the bicyclic and tricyclic systems would be too small to give good activity. In the present study, the synthesis and *in vitro* cytotoxic activities of 1,3-dioxoindan-2-carboxylic acid arylamides (**3**) are described (Fig. 1). The target compound contained the A, C, and D rings of the TAS-103 (**2**), but the B-ring was disconnected. Substituents on the phenyl ring system were introduced to explore the structure activity relationship.

MATERIALS AND METHODS

The melting points were measured on an Electro-thermal IA9100 digital melting point apparatus, and are

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uncorrected. The IR spectra were recorded with a Jasco FT/IR-300E spectrophotometer, and reported in cm^{-1} . $^1\text{H-NMR}$ spectra were recorded on Bruker DPS300 NMR spectrometer, using TMS as an internal standard, and the chemical shifts are reported as δ ppm units. Commercially available reagents and solvents were used without additional purification unless otherwise stated. The RPMI1640 media was obtained from Gibco BRL. Dimethyl sulfoxide (DMSO) and other chemicals were purchased from Sigma.

1,3-Dioxoindan-2-carboxylic acid ethyl ester (5)

Na (1.75 g, 76 mmol) was added to dry diethylphthalate (4) (10 g, 45 mmol). The mixture was heated to 115°C . Ethyl acetate (10 mL) was added and the reaction mixture stirred at 90°C for 7 h. After the reaction, ethanol (15 mL) was added and the mixture heated. After cooling, the solid part was filtered off and washed with ethanol to yield the Na salt (5.07 g) of 1, 3-dioxoindan-2-carboxylic acid ethyl ester (5). The Na salt (5.07 g) was diluted with hot water (50 mL) and filtered. The filtrate was acidified with dil. HCl solution and the precipitate filtered to give 5 (2.74 g, 28 %) as yellow solid; mp $77\text{--}78^\circ\text{C}$; IR (KBr) 1711, 1569, 1283 cm^{-1} ; $^1\text{H-NMR}$ (CD_3OD , 300 MHz) δ 7.48 (m, 4H, Ar-H), 4.27 (q, $J = 7.0$ Hz, 2H, CH_2), 1.33 (t, $J = 7.0$ Hz, 3H, CH_3).

General procedure for 1,3-dioxoindan-2-carboxylic acid arylamides (3)

To a suspension of 1, 3-dioxoindan-2-carboxylic acid ethyl ester (5) (2 g, 9.17 mmol) in toluene (80 mL) was added arylamine (10.09 mmol). The mixture was heated under reflux for 18 h. The reaction mixture was concentrated *in vacuo* and the residue was purified by recrystallization from ethanol to give 1,3-dioxoindan-2-carboxylic acid arylamides.

1,3-Dioxoindan-2-carboxylic acid phenylamide (3a)

The product was obtained in 17% yield as a dark green solid; mp $207\text{--}215^\circ\text{C}$; IR (KBr) 3236, 3050, 1658, 1587, 1544 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , 300 MHz) δ 9.28 (brs, 1H, NH), 7.87 (m, 2H, Ar-H), 7.70 (m, 4H, Ar-H), 7.43 (m, 3H, Ar-H).

1,3-Dioxoindan-2-carboxylic acid (2-hydroxyphenyl) amide (3b)

The product was obtained in 11% yield as a dark green solid; mp $> 300^\circ\text{C}$ (dec); IR (KBr) 3365, 3058, 1670, 1530 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , 300 MHz) δ 10.55 (s, 1H, OH), 9.08 (brs, 1H, NH), 7.86 (m, 3H, Ar-H), 7.63 (m, 3H, Ar-H), 7.00 (m, 2H, Ar-H).

1,3-Dioxoindan-2-carboxylic acid (2-methoxyphenyl) amide (3c)

The product was obtained in 11% yield as a dark green

solid; mp $131\text{--}132^\circ\text{C}$; IR (KBr) 3378, 3030, 1664, 1520 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , 300 MHz) δ 9.29 (brs, 1H, NH), 7.86 (m, 3H, Ar-H), 7.71 (m, 2H, Ar-H), 7.15 (m, 1H, Ar-H), 6.98 (m, 1H, Ar-H), 6.78 (m, 1H, Ar-H), 3.98 (s, 3H, OCH_3).

1,3-Dioxoindan-2-carboxylic acid (2-methylphenyl) amide (3d)

The product was obtained in 27% yield as a dark green solid; mp $253\text{--}255^\circ\text{C}$; IR (KBr) 3252, 3027, 1646, 1486 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , 300 MHz) δ 9.28 (brs, 1H, NH), 7.86 (m, 1H, Ar-H), 7.78 (m, 1H, Ar-H), 7.67 (m, 2H, Ar-H), 7.41–7.20 (m, 3H, Ar-H), 7.11 (m, 1H, Ar-H), 2.37 (s, 3H, CH_3).

1,3-Dioxoindan-2-carboxylic acid (4-methylphenyl) amide (3e)

The product was obtained in 35% yield as a dark green solid; mp $185\text{--}187^\circ\text{C}$; IR (KBr) 3273, 3037, 1647, 1487 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , 300 MHz) δ 9.26 (brs, 1H, NH), 7.87–7.68 (m, 4H, Ar-H), 7.46–7.29 (m, 4H, Ar-H), 2.37 (s, 3H, CH_3).

1,3-Dioxoindan-2-carboxylic acid (2,5-dimethoxyphenyl) amide (3f)

The product was obtained in 29% yield as a dark green solid; mp $182\text{--}183^\circ\text{C}$; IR (KBr) 3387, 1656, 1590, 1509 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , 300 MHz) δ 9.31 (brs, 1H, NH), 7.87 (m, 5H, Ar-H), 6.90 (m, 1H, Ar-H), 6.70 (m, 1H, Ar-H), 3.94 (s, 6H, OCH_3).

1,3-Dioxoindan-2-carboxylic acid (2,5-dichlorophenyl) amide (3g)

The product was obtained in 18% yield as a dark green solid; mp $198\text{--}200^\circ\text{C}$; IR (KBr) 3375, 1718, 1683, 1569, 1456 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , 300 MHz) δ 9.70 (brs, 1H, NH), 8.00 (m, 3H, Ar-H), 7.82 (m, 4H, Ar-H).

1,3-Dioxoindan-2-carboxylic acid (2,5-dimethylphenyl) amide (3h)

The product was obtained in 27% yield as a dark green solid; mp $240\text{--}242^\circ\text{C}$; IR (KBr) 3280, 3015, 2915, 1645, 1588 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , 300 MHz) δ 9.29 (brs, 1H, NH), 7.88–7.67 (m, 5H, Ar-H), 7.20 (m, 1H, Ar-H), 7.04 (m, 1H, Ar-H), 2.42 (s, 3H, CH_3), 2.33 (s, 3H, CH_3).

1,3-Dioxoindan-2-carboxylic acid (3,5-dichlorophenyl) amide (3i)

The product was obtained in 20% yield as a dark green solid; mp $287\text{--}290^\circ\text{C}$; IR (KBr) 3265, 3073, 1665, 1588, 1550 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , 300 MHz) δ 9.14 (brs, 1H, NH), 7.84 (m, 2H, Ar-H), 7.72 (m, 2H, Ar-H), 7.58 (m, 2H, Ar-H), 7.21 (m, 1H, Ar-H).

1,3-Dioxoindan-2-carboxylic acid (3,5-dimethylphenyl) amide (3j)

The product was obtained in 22% yield as a dark green solid; mp 218–220; IR (KBr) 3324, 3062, 1676, 1659, 1577, 1493 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , 300 MHz) δ 9.69 (brs, 1H, NH), 8.05–7.82 (m, 6H, Ar-H), 6.88 (s, 1H, Ar-H), 2.39 (s, 6H, CH_3).

1,3-Dioxoindan-2-carboxylic acid (3,5-bis(trifluoromethyl)phenyl)amide (3k)

The product was obtained in 13% yield as a dark green solid; mp 202–204°C; IR (KBr) 3411, 1718, 1683, 1569 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , 300 MHz) δ 9.70 (brs, 1H, NH), 7.96–7.77 (m, 7H, Ar-H).

Cells

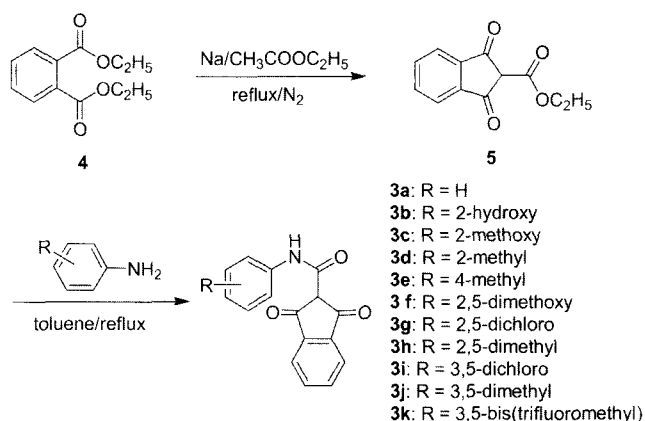
Four human cancer cell lines, HCT15, SK-OV-3, MD-MB-468 and T-47D, were used in this study. SK-OV-3 and HCT15 were obtained from the National Cancer Institute, U.S.A. MD-MB-468 and T-47D were purchased from the American Type Culture Collection. These cells were maintained in Dulbecco's modified eagle media, supplemented with 10% fetal calf serum, at 37°C under a humidified atmosphere of 5% CO_2 .

In vitro cytotoxicity assay

Cell numbers were measured indirectly by the sulforhodamine B (SRB) method, according to the NCI (USA)'s protocol (Skehan *et al.*, 1990). Briefly, cells were plated into 96 well plate at a density of 2×10^3 cells per well. The following day (day 0), the compounds of interest, dissolved in DMSO/media, were added in quadruplicate. The final concentrations of each compound were 1 nM–10 μM and the final concentration of DMSO was <0.1%. 72 hours later, cells were fixed with 10% trichloroacetic acid (TCA) overnight at 4°C. The TCA-treated cells were extensively washed with distilled water and dried in the air. The SRB solution (0.4% in 1% acetic acid) was then added to each well at room temperature for one hour. The bound dye was solubilized with 10 mM Tris after washing the wells with 1% acetic acid, and the absorbances were measured at 690 nm using a microplate reader. The absorbance value of day 0 was subtracted from the absorbance values obtained at day 3.

RESULTS AND DISCUSSION

The syntheses of the target compounds (**3a–k**) are outlined in Scheme 1. 1,3-Dioxoindan-2-carboxylic acid ethyl ester (**5**) was prepared according to the reported procedure (Vanags *et al.*, 1946). Diethyl phthalate (**4**) was treated with Na and ethyl acetate to give compound (**5**). Compound **5** was then treated with various amines (aniline,



Scheme 1. Synthesis of 1,3-dioxoindan-2-carboxylic acid *N*-arylamides

2-hydroxyaniline, 2-methoxyaniline, 2-methylaniline, 4-methylaniline, 2,5-dimethoxyaniline, 2,5-dichloroaniline, 2,5-dimethylaniline, 3,5-dichloroaniline, 3,5-dimethylaniline, and 3,5-bis(trifluoromethyl)aniline) under reflux in toluene to give compounds **3a–k** in yields from 11 to 35%. The structures of the target compounds were assigned from the spectroscopic data.

Evaluations of the biological activities for the compounds were performed *in vitro* following the protocols developed by the National Cancer Institute (Skehan *et al.*, 1990). The *in vitro* cytotoxic activities of the 1,3-dioxoindan-2-carboxylic acid arylamides (**3a–k**) against human cancer cell lines originating from colon (HCT-15), ovarian (SK-OV-3), breast adenocarcinoma (MD-MB-468) and breast ductal carcinoma (T-47D), along with comparative data for doxorubicin and mitomycin-C, are listed in Table I.

The target compounds were designed to have the A, C and D ring systems of TAS-103 (**2**), with the B-ring of TAS-103 disconnected and the bicyclic C and D-rings of TAS-103 linked to the A-ring through an amide bond. Various substituents were introduced into the *N*-phenyl ring system (A-ring) to delineate the structure activity relationship. In the 2-substituted series of derivatives, compound **3c**, containing a methoxy group, was 5 to 25 times more potent against the HCT15 cell line than the compounds having the 2-hydroxy (**3b**) and 2-methyl (**3d**) substituents. The 2,5-disubstituted derivatives (**3f**, **3g** and **3h**) exhibited similar cytotoxic activities. In the case of the 3,5-disubstituted series of derivatives, compound **3i** containing dichloro substituents was 5 to 20 times more potent against the HCT15 cell line than the compounds having the 3,5-dimethyl (**3j**) and 3,5-bis(trifluoromethyl) (**3k**) substituents. Among these analogs, the most active was compound **3e** (1.2 μM against SK-OV-3 cell line) bearing the 4-methyl substituent.

The target compounds showed more potent cytotoxic activities than our previous 2-substituted 4-methylquinoline derivatives (Lee *et al.*, 2000) and cyclopentaquinoline

Table I. *In vitro* cytotoxic activity of 1,3-dioxindan-2-carboxylic acid *N*-arylamides

Cpd No.	R	IC ₅₀ (μM)			
		HCT15	SK-OV-3	MD-MB-468	T-47D
3a	H	25	30	20	13
3b	2-hydroxy	8	30	20	24
3c	2-methoxy	1.6	6	3.1	5.3
3d	2-methyl	40	13	10	3.3
3e	4-methyl	1.7	1.2	4.1	4
3f	2,5-dimethoxy	7	17	30	44
3g	2,5-dichloro	22	>100	20	22
3h	2,5-dimethyl	20	20	15	12
3i	3,5-dichloro	1.4	20	5	3
3j	3,5-dimethyl	7	34	3.6	11
3k	3,5-bis(trifluoromethyl)	30	>100	22	34
Doxorubicin		0.022	0.012	0.002	0.005
Mitomycin-C		0.05	0.12	0.03	0.4

^aIC₅₀=Concentration of compound (μM) required to inhibit the cellular growth by 50% after 72 h of drug exposure, as determined by the SRB assay. Each experiment was run at least three times, and the results are presented as the average value. ^bHuman cancer cell lines: HCT15 (colon cancer cell), SK-OV-3 (ovarian cancer cell), MD-MB-468 (human breast adenocarcinoma) and T-47D (human breast ductal carcinoma).

derivatives (Lee *et al.*, 2001) that were designed as truncate analogues of TAS-103. However, these were generally less potent than doxorubicin and mitomycin-C. This result suggests that an additional ring system may be necessary for good activity. Work is in progress to design, synthesize and evaluate additional compounds in this and related systems.

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