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ANTIMICROBIAL AND ANTIVIRAL ACTIVITY OF SPIROINDOLINONES BEARING BENZOTHIAZOLE MOIETY

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SUMMARY

In this study, 5-chloro-1'-methyl-5'-nitro-3*H*-spiro[1,3-benzothiazole-2,3'-indole]-2'(1'*H*)-one (**3u)** was synthesized by the reaction of 1-methyl-5-nitro-1*H*-indole-2,3-dione (**1m)** with 2-amino-4-chlorothiophenol **(2)** in ethanol. The structure of **3u** was confirmed by the spectral (IR, ^{1}H NMR, HSQC-2D, LCMS-ESI) data and elemental analysis. The new spiroindolinone derivative **3u**, along with previously reported spiroindolinone derivatives **3a-t** bearing benzothiazole or 5-chlorobenzothiazole moiety were tested for *in vitro* antimicrobial activity against selected strains. Among the tested compounds, **3i** and **3l** displayed the highest efficacy against *Staphylococcus aureus* and *Candida albicans*. Only **3b** was found to be significantly active against *Staphylococcus epidermidis*. **3a-n** were evaluated for *in vitro* antituberculosis activity against *Mycobacterium tuberculosis* H37Rv, but most of the tested compounds showed weakly antitubercular activity. All compounds were also evaluated against some DNA and RNA viruses in CRFK, HeLa and HEL cells. Cytotoxicities of the tested compounds were generally very high compared to standards.

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ÖZET

Bu çalışmada 1-metil-5-nitro-1*H*-indol-2,3-dion (**1m)** ile 2-amino-4-klorotiyofenol **(2)**'ün etanollü ortamdaki reaksiyonundan 5-kloro-1'-metil-5'-nitro-3*H*-spiro[1,3-benzotiyazol-2,3'-indol]-2'(1'*H*)-on (**3u)** bileşiği sentezlenmiştir. **3u** nun yapısı spektral (IR, ^{1}H NMR, HSQC-2D, LCMS-ESI) bulgular ve elemental analiz ile kanıtlanmıştır. Yeni spiroindolinon türevi **3u**, daha önce rapor edilen benzotiyazol ya da 5-klorobenzotiyazol artığı taşıyan spiroindolinon türevleri **3a-t** ile birlikte seçilen bakteri suşlarına karşı *in vitro* antibakteriyel aktivite için test edilmiştir. Test edilen bileşikler içinde **3i** ve **3l** *Staphylococcus aureus* ve *Candida albicans*'a karşı en yüksek etkinlik göstermiştir. Yalnız **3b** *Staphylococcus epidermidis'* e karşı önemli derecede etkili bulunmuştur. **3a-n** nin *Mycobacterium tuberculosis* H37Rv karşı *in vitro* antitüberküloz etkileri incelenmiştir; ama test edilen bileşiklerin çoğu zayıf antitüberküloz etki göstermiştir. Tüm bileşikler CRFK, HeLa ve HEL hücrelerinde DNA ve RNA virüslerine karşı ayrıca test edilmiştir. Test edilen bileşiklerin sitotoksisiteleri standartlara kıyasla genellikle çok yüksektir.

Key words: Spiroindolinones, benzothiazole, antimicrobial activity, antituberculosis activity, antiviral activity.

INTRODUCTION

1*H*-Indole-2,3-dione (isatin) is a naturally occurring product found in several plant species, such as *Isatis tinctoria*, *Calanthe discolor* and *Couroupita guianensis*. In humans, it is found as a metabolic derivative of adrenaline (1). Isatin and its derivatives have wide usage in medicinal chemistry due to their easy availability and their usability as both electrophilic and nucleophilic agents. Ligands that are based on these scaffolds show a diverse spectrum of antimicrobial, antiviral and antitumor activities (2,3). Spiroindolinones are also alkaloids obtained from *Horsfieldia superba* (for example horsfiline) and *Eleagnus commutata* (for example elacomine). Similarly, these compounds are reported to exhibit broad spectrum chemotherapeutic properties (Figure 1) (3,4).

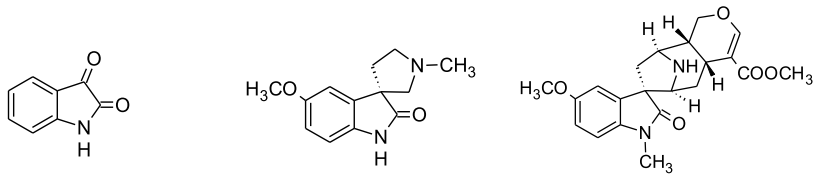


Figure 1. Chemical structures of isatin (a), (-)-horsfiline (b) and (elacomine (c)

In our previous studies, several spiroindolinone derivatives **3a-t** incorporating benzothiazole or 5-chlorobenzothiazole nucleus were synthesized and evaluated for anticancer and antioxidant activities (5-7). In the current study, a new spiroindolinone compound **3u** was synthesized. Subsequently, previously reported compounds **3a-t** and compound **3u** were evaluated for antimicrobial and antiviral activities.

MATERIAL AND METHODS

General procedures

Melting points were estimated with a Buchi 540 melting point apparatus in open capillaries and were uncorrected. Elemental analyses were performed on a Thermo Finnigan Flash EA 1112 elemental analyzer. IR spectra were recorded on KBr discs, using a Perkin-Elmer Model 1600 FT-IR spectrometer. ^{1}H-NMR and HSQC spectra were obtained on VarianUNITY INOVA 500 spectrophotometers using DMSO-d_6. Mass spectra were determined on Finnigan TM LCQ TM and AGILENT 1100 MSD instruments.

The synthesis of 1-methyl-5-nitro-1H-indole-2,3-dione **(1m)**

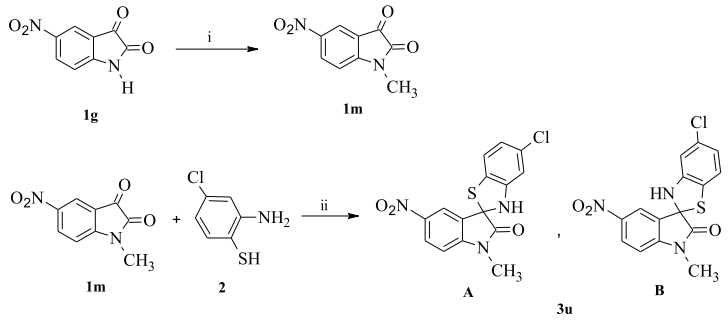
A suspension of 5-nitro-1*H*-indole-2,3-diones **1g** (5 mmol) and NaH (50% dispersion in mineral oil) (0.2 g) in anhydrous DMF (5 mL) was stirred for 30 min. at room temperature. After addition of iodomethane (15 mmol), the mixture was refluxed for 4 h. The product was poured onto ice and water, and subsequently filtered.

The synthesis of 5-chloro-1'-methyl-5'-nitro-3H-spiro[1,3-benzothiazole-2,3'-indole]-2'(1'H)-one **(3u)**

To a solution of 1-methyl-5-nitro-1*H*-indole-2,3-dione **1m** (3.5 mmol) in ethanol (15 mL) was added 2-amino-4-chlorothiophenol **2** (3.5 mmol). The mixture was refluxed on a water bath for 4 h. The product formed after cooling was filtered and recrystallized from ethanol. Yield 43%; m.p. 122-126 ˚C; IR (KBr) cm^{-1}: υ 3349 (NH), 1739 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) d (ppm): 3.20, 3.21 (3H, 2s, N-CH_3); 6.57, 6.69 (1H, 2dd, *J*=8.78, 1.95 Hz, benzothia. C_6-H); 6.63, 6.76 (1H, 2d, *J*=1.95 Hz, benzothia. C_4-H); 7.09, 7.11 (1H, 2d, *J*=8.29 Hz, benzothia. C_7-H); 7.29, 7.34 (1H, 2d, *J*=8.79 Hz, ind. C_7-H); 7.52 (1H, s, benzothia. NH); 8.22, 8.30 (1H, 2d, *J*=2.44 Hz, ind. C_4-H); 8.35, 8.53 (1H, 2dd, *J*= 8.78; 2.44 Hz, ind. C_6-H). HSQC-2D (DMSO-d_6/TMS) d (ppm): 27.98, 27.99 (ind. 5-CH_3), 75.12 (spiro C), 109.45, 109.51 (benzothia. C_4), 109.48, 110.91 (ind. C_7), 118.96, 120.10 (benzothia. C_6), 121.47 (ind. C_4), 123.42 (benzothia. C_7), 123.94 (ind. C_{3a}), 128.76, 134.13 (ind. C_6), 131.37 (benzothia. C_5), 131.67 (benzothia. C_{7a}), 144.28 (ind. C_5), 149.07 (ind. C_{7a}), 149.90 (benzothia. C_{3a}), 175.83, 182.39 (ind. C=O). LCMS-ESI (+) m/z (%): 348, 349 (MH^+; 31, 12); 346, 348 (67, 31); 345, 347 (100, 48); 344, 346 (75, 67). Analyses (%) Cald for $C_{15}H_{10}ClN_3O_3S$ (347.78): C, 51.08; H, 2.90; N, 12.08. Found: C, 51.02; H, 2.86; N, 11.59.

RESULTS AND DISCUSSION

5-Chloro-1'-methyl-5'-nitro-3*H*-spiro[1,3-benzothiazole-2,3'-indole]-2'(1'*H*)-one **3u** was synthesized by the reaction of 1-methyl-5-nitro-1*H*-indole-2,3-dione **1m** with 2-amino-4-chlorothiophenol **2** in ethanol (Scheme 1) (5-7). The structure of **3u** was confirmed by spectral (IR, ^{1}H NMR, HSQC-2D, LCMS-ESI) data and elemental analysis.



Scheme 1. Preparation of spiroindolinone derivative 3u. Reagents and conditions: i) NaH, anhyd.

DMF, stirred, 0.5h, CH_3I, reflux, ii) EtOH, reflux 4 h.

IR spectra of **3u** showed absorption bands in the 3349 and 1739 cm^{-1} regions resulting from the NH and C=O functions, respectively [1, 8]. ^1H NMR spectra of **3u** displayed the NH proton of the benzothiazole (δ 7.52 ppm) ring as a separate singlet [9]. The indole NH resonances were not observed and thus the structure of **3u** was assigned to be the R_2-methylated derivative. The spectra of **3u** supported this finding as it displayed the R_2-methyl resonances (δ 3.20 and 3.21 ppm). No duplication of signals were observed in the NMR spectra of **3a-t** whereas the resonances were observed as double signals in **3u**. Duplicate signals may originate from unique molecules, *A* and *B*, in the asymmetric unit. The benzothiazole rings in both molecules adopt an envelope conformation. The indolinone rings in both molecules are also not planar, with a twisted conformation. In the crystal structure, there are intermolecular N—H··O and N—H··S hydrogen-bonding interactions. The X-ray data of **3i** was determined in order to confirm the assigned spiro structures (6). The formation of spiroindolinone is evident by the presence of a signal assigned to spiro C (δ 75.12 ppm) and the presence of signals attributed to the C=O function of indolinone (δ 175.83 and 182.39 ppm) in the HSQC spectra of **3u** [10]. In the mass spectra of **3u** showed MH^+ and MH^++2 peaks which confirmed their molecular weights.

The antimicrobial activities of compound **3u**, along with previously reported compounds **3a-t** were determined by the microbroth dilutions technique using the Clinical Laboratory Standards Institute (CLSI) recommendations against *Staphylococcus aureus ATCC 6538*, *Staphylococcus epidermidis ATCC 12228*, *Escherichia coli ATCC 25922*, *Klebsiella pneumoniae ATCC 4352*, *Pseudomonas aeruginosa ATCC 27853, Proteus mirabilis ATCC 14153* and *Candida albicans* ATCC 10231. Ciprofloxazin, clotrimazole and flukonazol were used as the standards in the tests (Table 1) (11,12). None of the test compounds was active against *E. coli ATCC 25922*, *K. pneumoniae ATCC 4352*, *P. mirabilis ATCC 14153* (>5000 μg/ml), whereas compounds **3a-u** have considerable antimicrobial effect on *S. aureus, S. epidermidis* and *C. albicans*. Among the tested compounds, the most active compounds against *S. aureus* were R_1-nonsubstituted **3i** (4.9 μg/ml) and R_1-fluor substituted **3l** (4.9 μg/ml). These compounds displayed the highest activity against *C. albicans,* too. The microbial inhibition concentrations (MIC) against *C. albicans* of both compounds were

15.6 µg/ml and 19.5 µg/ml, respectively. R_2-nonsubstituted compounds, **3i** and **3l,** have a chlorine atom at the R_3 position. Only R_1-chlorine substituted **3b** showed significantly activity against *S. epidermidis* (4.9 µg/ml). The preliminary screening results indicated that the substitution of the chlorine at R_3 caused significantly increase in the activity against *C. albicans*, whereas none of the R_3-chlorine substituted compounds was active against *S. epidermidis.* The activities of tested compounds were lower compared to the activities of the standard compounds.

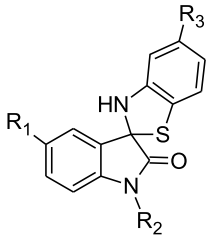


Table 1. The microbial inhibition concentrations (MIC) of **3a-u** against three bacterial strains.

	MIC (µg/ml)					
Compound	**R_1**	**R_2**	**R_3**	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Candida albicans*
3a	CH_3	H	H	39	n.a.	n.a.
3b	Cl	H	H	9.8	4.9	n.a.
3c	NO_2	H	H	19.5	39	n.a.
3d	CH_3	CH_3	H	78	n.a.	n.a.
3e	CF_3O	CH_3	H	19.5	n.a.	n.a.
3f	Cl	CH_3	H	39	625	n.a.
3g	Br	CH_3	H	n.a.	n.a.	n.a.
3h	NO_2	CH_3	H	312	n.a.	n.a.
3i	H	H	Cl	4.9	n.a.	15.6
3j	CH_3	H	Cl	n.a.	n.a.	n.a
3k	CF_3O	H	Cl	n.a.	n.a.	n.a
3l	F	H	Cl	4.9	n.a.	19.5
3m	Cl	H	Cl	n.a.	n.a.	n.a.
3n	Br	H	Cl	19.5	n.a.	312
3o	NO_2	H	Cl	n.a.	n.a.	n.a.
3p	CH_3	CH_3	Cl	n.a.	n.a.	312
3q	CF_3O	CH_3	Cl	n.a.	n.a.	78
3r	F	CH_3	Cl	n.a.	n.a.	312
3s	Cl	CH_3	Cl	n.a.	n.a.	312
3t	Br	CH_3	Cl	n.a.	n.a.	312
3u	NO_2	CH_3	Cl	n.a.	n.a.	312
Ciprofloxacine				0.25	0.125	n.t.
Clotrimazole				n.t.	n.t.	4.9
Flukonazol				n.t.	n.t.	1

n.a.: not active (> 5000 µg/ml); n.t.: not tested

Compounds **3a-u** were evaluated against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA). The primary antituberculosis screening was performed in accordance with the protocol of the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) Southern Research Institute (13). Rifampin was used as the control drug in the tests. Compounds demonstrating a percent inhibition of bacterial growth of greater than or equal to 90% in the primary screen were retested against *M. tuberculosis* H37Rv, to determine the actual minimum inhibitory concentration (MIC) in the MABA. The MIC was defined as the lowest concentration effecting a reduction in fluorescence of 90%, relative to controls. This value was determined from the dose-response curve as the IC_{90} using a curve fitting program. Any IC_{90} value of $\leq$ 10 µg/mL was considered ‘‘Active" for antitubercular activity. Compounds active in the initial screen were tested for cytotoxicity (IC_{50}) in VERO cells. Cytotoxicity was determined from the dose-response curve as the IC_{50} using a curve fitting program. Concurrent with the determination of MICs, compounds were tested for cytotoxicity in VERO cells at concentrations 10x the MIC for *M. tuberculosis* H37Rv. Most of the tested compounds showed weakly antitubercular activity and cytotoxicities of the compounds were found to be very high (Table 2).

Table 2. Primary *in vitro* antimycobacterial activity of **3a-u** against *M. tuberculosis* H37Rv.

	H37Rv Data			
Compound	**Assay**	**Activity**	**IC_{50} (µg/mL)**	**IC_{90} (µg/mL)**
3a	MABA	Weak Active	74.677	>100
3b	MABA	Weak Active	36.269	77.361
3c	MABA	Inactive	>100	>100
3d	MABA	Weak Active	46.785	55.402
3e	MABA	Weak Active	>100	>100
3f	MABA	Weak Active	48.518	85.114
3g	n.t.	n.t.	n.t.	n.t.
3h	MABA	Inactive	>100	>100
3i	MABA	Weak Active	27.58	95.652
3j	n.t.	n.t.	n.t.	n.t.
3k	MABA	Weak Active	13.676	21.592
3l	MABA	Weak Active	45.064	71.685
3m	MABA	Weak Active	25.507	29.645
3n	MABA	Weak Active	22.024	30.857
3o	n.t.	n.t.	n.t.	n.t.
3p	n.t.	n.t.	n.t.	n.t.
3q	n.t.	n.t.	n.t.	n.t.
3r	n.t.	n.t.	n.t.	n.t.
3s	n.t.	n.t.	n.t.	n.t.
3t	n.t.	n.t.	n.t.	n.t.
3u	n.t.	n.t.	n.t.	n.t.
Rifampin				0.125

n.t.: not tested

The compounds **3a-u** were also evaluated against feline corona virus (FIPV), feline herpes virus (FHV) in Crandell-Rees feline kidney (CRFK), herpes simplex virus-1 (KOS)(HSV-1), herpes simplex virus-2 (G) (HSV-2), vaccinia virus, vesicular stomatitis virus (VSV), herpes simplex virus-1 TK KOS ACV in human embroyonic lung (HEL) and vesicular stomatitis virus in Henrietta Lacks (HeLa) cell cultures. Brivudin, ribavirin, cidofovir and ganciclovir were used as the standards in the tests (Table 3) (14). The most active compound was R_1-bromo substituted **3n**. EC_{50} values of **3n** were >0.16 μM for all tested virus strains (herpes simplex virus-1 (KOS) (HSV-1), herpes simplex virus-2 (G) (HSV-2), vaccinia virus, vesicular stomatitis virus (VSV) and herpes simplex virus-1 TK KOS ACV). R_1-trifluoromethoxy substituted **3k**, R_1-chloro substituted **3m**, R_1-nitro substituted **3o** and R_1-trifluoromethoxy substituted **3q** showed EC_{50} values of >0.8 μM for the same virus strains (Table 3). Most of these compounds (**3k**, **3m**, **3n** and **3o)** were R_2- nonsubstituted compounds incorporating a chlorine atom at position R_3, whereas **3q** was R_2- methyl substituted a compound. However, minimum cytotoxic concentrations (mM) of these compounds were very high compared to standards.

Table 3. Antiviral and cytotoxic effects (EC_{50}) of **3a-u** against some RNA and DNA viruses.

Compound	Minimum cytotoxic concentration[a] (μM)	EC_{50} (μM)[b]				
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 TK- KOS ACV
3a	≥20	>20	>20	>20	>20	>20
3b	20	>4	>4	>4	>4	>4
3c	20	>4	>4	>4	>4	>4
3d	100	>20	>20	>20	>20	>20
3e	≥20	>20	>20	>20	>20	>20
3f	≥20	>20	>20	>20	>20	>20
3g	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
3h	≥20	>20	>20	>20	>20	>20
3i	≥20	>20	>20	>20	>20	>20
3j	100	>20	>20	>20	>20	>20
3k	4	>0.8	>0.8	>0.8	>0.8	>0.8
3l	≥4	>4	>4	>4	>4	>4
3m	≥0.8	>0.8	>0.8	>0.8	>0.8	>0.8
3n	0.8	>0.16	>0.16	>0.16	>0.16	>0.16
3o	4	>0.8	>0.8	>0.8	>0.8	>0.8
3p	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
3q	4	>0.8	>0.8	>0.8	>0.8	>0.8
3r	20	>4	>4	>4	>4	>4
3s	20	>4	>4	>4	>4	>4
3t	20	>4	>4	>4	>4	>4
3u	20	>4	>4	>4	>4	>4
Brivudin	>250	0.04	50	0.8	>250	250
Ribavirin	>250	112	>250	50	250	250
Cidofovir	>250	1.2	0.4	5	>250	1
Ganciclovir	>100	0.03	0.1	>100	>100	4

n.t.: not tested; a: required to cause a microscopically detectable alteration of normal cell morphology; b: required to reduce virus-induced cytopathogenecity by 50 %

Against Feline corona virus (FIPV) and Feline herpes virus, compounds **3i**, **3n** and **3o** showed EC_{50} values of higher than >0.8 μM (Table 4). These compounds have a hydrogen atom at position R_2 and a chlorine atom at position R_3. The substitution of the chlorine group at R_3 usually increased the activities of compounds against Feline corona and Feline herpes viruses. However, cytotoxicities of all compounds were very high compared to standards. HHA lectin, UDA lectin and ganciclovir were used as the standards in the tests.

Table 4. Antiviral and cytotoxic effects of **3a-u** against Feline corona and Feline herpes viruses.

Compound	Minimum cytotoxic concentration[a] (μM)	EC_{50} (μM)[b]	
		Feline corona virus (FIPV)	Feline herpes virus
3a	>100	>100	>100
3b	44.0	>20	>20
3c	27.8	>20	>20
3d	54.9	>20	>20
3e	56.5	>20	>20
3f	45.1	>20	>20
3g	n.t.	n.t.	n.t.
3h	>100	>100	>100
3i	2.3	>0.8	>0.8
3j	>100	>100	>100
3k	>100	>100	>100
3l	>100	>100	>100
3m	>100	>100	>100
3n	3.2	>0.8	>0.8
3o	2.1	>0.8	>0.8
3p	n.t.	n.t.	n.t.
3q	12	>4	>4
3r	9.1	>4	>4
3s	58	>20	>20
3t	10	>4	>4
3u	8.2	>4	>4
HHA lectin	>100	8.9	5.9
UDA lectin	>100	54.1	6.8
Ganciclovir	>100	>100	5.2

n.t.: not tested; a: required to cause a microscopically detectable alteration of normal cell morphology; b: required to reduce virus-induced cytopathogenecity by 50 %

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