Chemical and Pharmaceutical Bulletin Advance Publication by J-STAGE
DOI:10.1248/cpb.c17-00409

Advance Publication
July 22, 2017

Chem. Pharm. Bull

Note

5,6-dihydropyrrolo[2,1-*a*]isoquinolines as alternative of new drugs with cytotoxic activity

Rosa Ma. Chávez-Santos,[a] Paul E. Reyes-Gutiérrez,[b] Rubén O. Torres-Ochoa,[b]
Ma. Teresa Ramírez-Apan[a], Roberto Martínez,[*a]
[a] *Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, 04510, Cd. México, México.* [b]*Institute of Organic Chemistry and Biochemistry, CAS; Flemingovo náměstí 542/2, 16610 Prague 6, Czech Republic.*

* robmar@unam.mx

Abstract

In this study, the pyrrolo[2,1-*a*]isoquinolines **4a-n** were synthesized in good yields in a three steps synthesis from the corresponding α,β-unsaturated esters starting materials. These compounds were tested on six human cancer cells lines to measure the cytotoxic activity as a function of the electronic properties and aromaticity of the substituent at the C-2 position of the pyrroloisoquinoline. Our results reveal that the cytotoxic activity could be explained in terms of the distribution of electronic density across the ring joined to C-2. Also, this study identified 3-hydroxy (**4d)** and 3-chloro (**4j)** derivatives with powerful cytotoxic activities The IC_{50} values of these compounds were found to be comparable to those of the commercially available Topotecan, Irinotecan, Etoposide, Tamoxifen, and Cisplatin.

Keywords: pyrrolo[2,1-*a*]isoquinolines; synthesis, cytotoxic activity.

Introduction

Cancer is a leading cause of death worldwide, and according to the World Cancer Research Fund International (GLOBOCAN 2012), an estimated 17.1 million new cancer cases and 10.0 million cancer-related deaths will occur in 2020, compared with 15.2 million and 8.9 million, respectively, in 2015.[1)] The most commonly diagnosed cancers worldwide are cancers of the skin (melanoma), prostate, lung, breast, and colorectum.[2)] Cancer poses a challenge to researchers searching for potent drugs that are capable of controlling cancer growth while minimizing side effects or the development of drug resistance. Our group is currently engaged in a program aimed at synthesizing novel heterocyclic compounds that inhibit the growth of cancer cells. We recently synthesized the pyrroloisoquinolines **I–IV** (Table 1)[3)] in a three-step protocol, and their cytotoxic activities were tested on six tumor cell lines: PC-3 (human prostatic adenocarcinoma), U-251 (human glioblastoma), K-562 (human chronic myelogenous leukemia), HCT-15 (human colorectal adenocarcinoma), MCF-7 (human mammary adenocarcinoma), and SKLU-1 (human lung adenocarcinoma). The results of this study (Table 1), allowed us to establish preliminary structure–activity relationship (Figure 1) that revealed the importance of the aromatic substituent at the C-2 position, particularly when the substituent was a *m*-(cyclohexylmethylpiperazinamide) phenyl (**I**), phenyl(**II**), or *m*-(amino)phenyl (**III**), substituent, in combination with an ethyl ester at the C-1 position. Following up on these preliminary structure–activity studies, the present work sought to synthesize the novel pyrroloisoquinolines **4a–c** (Figure 2) to evaluate the effects of modifying the cyclohexylmethylpiperazinyl moiety on the antiproliferative activity. Compounds **4d–k** (Figure 2) were evaluated to determine the role of the phenyl ring substituent on the antiproliferative activity of the compound. Compounds **4l–n** (Figure 2) were evaluated to determine the effect of modifying the aromaticity of the C-2 substituent on the antiproliferative activity.

Results and discussion

Chemistry

The synthetic route to compounds **4a-n** is depicted in Chart 1. Compounds **4a-n** were synthetized *via* a three-step procedure starting from α,β-unsaturated esters **1a',1b, 1c, 1d', 1e-j, 1k', 1l', 1m** and **1n** based in our previous synthetic strategy for synthesis of 5,6-pyrrolo[2,1-*a*]isoquinolines **I-IV** with slightly modifications.[3)] According with our synthetic

Chart 1, the reaction between the corresponding aromatic aldehydes and ethyl diethylphosphonoacetate, using a Horner–Wadsworth protocol[4] gave the starting materials **1c, 1d', 1e-j, 1l', 1m**, and **1n** in excellent yields (Chart 2). The ethyl cinnamates **1a'** and **1b** were obtained from *m*-iodobenzoic acid or *m*-iodoaniline through a Heck reaction[5] and a subsequent amidation reaction[6] with the corresponding cyclic amines (Chart 3). It should be noted that all the α,β-unsaturated esters prepared were obtained exclusively as the (*E*)-isomers in good yields (78–98%).

Treatment of the electrophilic alkenes **1a'–n** with monomethylated TosMIC, prepared from the commercially available TosMIC under phase transfer conditions according to van Leusen's protocol,[7] afforded the 2,3,4-polysubstituted pyrroles **2a'–n** in 70–96% yields. The key intermediates **3a'–n** were prepared by N-alkylation of the pyrroles **2a'–n** using 2-bromo-4,5-dimethoxyphenethyl 4-methylbenzenesulfonate as alkylating agent, which had been prepared from 2-(3,4-dimethoxyphenyl)ethanol in the presence of NaH as a base in dry DMSO. Finally, the *N*-alkyl-pyrroles **3a'–n** were cyclized to the corresponding tetrasubstituted-5,6-dihydropyrroloisoquinolines **4a–n** using radical oxidative conditions in the presence of tributyltin hydride (*n*-Bu_3SnH) and dilauroyl peroxide (DLP) in toluene.[8] The nitro derivative was prepared via a palladium-catalyzed reaction[9] used to obtain the tricyclic framework **4g**. This step was necessary due to the possibility of a denitration reaction in the presence of tributyltin hydride (Chart 1, conditions iii'). The piperazinyl compound **4a** was prepared from compound **4a'** in ethanol using hydrazine hydrate as a deformylating agent.[10] The pyrroloisoquinoline **4d** was prepared after hydrogenolysis of the benzyl ether **4d'**.[11] The bromo derivative **4k** was synthesized through a Sandmeyer reaction[12] using the corresponding aniline **4k'** as a precursor. Finally, the *N*-deprotection of pyrrole **4l'** was achieved after applying the reductive conditions described by Sajiko,[13] which afforded the pyrroloisoquinoline **4l.**

Cytotoxic Activity and Structure-Activity Relationship(SAR) for compounds 4a-4m

We evaluated the effects of modifying the cyclohexylmethylpiperazinyl group in compound **I** on the antiproliferative activities (Table 2). The removal of the methylcyclohexyl group from our lead compound **I** decreased the antiproliferative activity of the synthesized compound **4a** in all cell lines compared to the activity of the lead compound **I** [Table 2, Entry 3]. These results suggested that the methylcyclohexyl group is key to the cytotoxic activity. The influence of the piperazine NH group on the activity of **4a** was examined by synthesizing the morpholinyl analog **4b**. Surprisingly, **4b** did not inhibit proliferation of any of the six cancer lines tested (Table 2, Entry 4). On the other hand, the antiproliferative activity of *N*-formylpiperazine **4a'** displayed inhibition levels minor to those displayed by **4a**. These results could be attributed to effect of the piperazinyl-NH group of **4a** on the activity, probably by forming a quaternary ammonium ion *in situ*.

Complete removal of the piperazine ring, in compound **4c** (3-$CONH_2$), provided a level of growth inhibition in all cell lines tested that exceeded the inhibitory activity of **4a.** The antiproliferative activity of **4c** was better than that of the lead compound **I** in the MCF-7 cell line but lesser than in tested: PC-3, U-251, K-562, HCT-15 and SKLU-1 cell lines. The last results suggested that the electronic properties of amide group of compound (**4c)** affected the cytotoxicity to a greater degree than the electronic properties of cyclohexylmethyl-piperazinyl group on **I** in the MCF-7 cancer cell line.

The electronic effects were further examined by introducing electron-donating groups (**4d**, 3-OH; **4e**, 3-OMe) or electron-withdrawing groups (**4f**, 3-CN; **4g**, 3-NO_2; **4h**, 3-CF_3) at the *meta* position of the 2-benzene ring. The majority of these changes significantly increased the inhibitory activity compared to the unsubstituted compound **II** in all cell lines (Table 3). By the contrary, the inhibitory activity of compounds **4d-h** was lesser than that the lead

compound **I** in all cell lines, with exception in HCT-15 cell line were compound **4d** was twice times more active than **I**. Interestingly, compound **4d** (3-OH) was the most active derivative across three of the six cancer lines (Table 3). The nature of the –OH substituent appeared to increase the activity due to the capacity of the substituent to form hydrogen bonds.

The roles of the electronegativity and/or size of the halogen group on the antiproliferative activity were investigated by synthesizing compounds **4i** (3-F), **4j** (3-Cl), and **4k** (4-Br). As shown in Table 3, the chloro derivative **4j** was the most active of the halogenated compounds on all the cancer cell lines tested but was lesser than that the lead compound **I**, with exception in MCF-7 cell line were compound **4j** was six times more active than **I**. These results suggest that derivatization of C-2-phenyl group with an m-chloro substituent is suitable to obtain best anticancer pyrroloisoquinoline compounds

Finally, the noteworthy results that have been obtained by studying bioisosteric compounds[14] led us to synthesize bioisosters of **II** by changing the benzene ring to a pyrrolo, a furan or a pyridine ring, creating compounds **4l**, **4m**, and **4n**, respectively. Our results demonstrate that a bioisosteric modification of the C-2 benzene ring of compound **II**, gives compounds with preserved cytotoxic activity. Moreover, this activity is enhanced by the presence of a furan ring in the PC-3 cell line (compound **4m**). However, the inhibitory activity of compounds **4l-n** was lesser than that the lead compound **I** in all cell lines tested. Likewise, the data for compounds **II**, **IV**,**4l-m** in three of the six cancer lines U-251, HCT-15 and SKLU-1 indicate that the cytotoxic effect was dependent of the kind of aromaticity of the substituent joined to C-2. These findings confirmed that the cytotoxic activity could be explained in terms of the distribution of electronic density across the ring joined to C-2.

The cytotoxic activities of our most active compounds **4d** and **4j** were compared with those of the commercially available Topotecan,[15] Irinotecan,[16] Etoposide,[17] Tamoxifen,[18] and

Cisplatin.[19] As shown in Table 3, compound **4d** (3-OH) was ten times more active than cisplatin in the prostate PC-3 cell line, almost two thousand times more active than irinotecan in the colon HCT-15 cell line. In the lung SKLU-1 cell line, compound **4d** (3-OH) was thirty-seven times more active than topotecan, six hundred forty-one times more active than irinotecan and seventy-six times more active than etoposide. Compound **4j** (3-Cl) showed more activity in the U-251(CNS) cell line and leukemia K-562 cell line compared to the reference etoposide. Finally, compound **4j** (3-Cl) showed a higher activity than tamoxifen in the breast cancer cell line MCF-7

Conclusion

Compounds **4a–4n** were synthetized trough a practical synthetic route involving the van Leusen's pyrrole construction protocol and an intramolecular radical oxidative cyclization. The inhibitory activities of compounds **4a–4n** were evaluated using six cancer cell lines. Our results reveal that the cytotoxic activity could be explained in terms of the distribution of electronic density across the ring joined to C-2. Also, the present study enabled the discovery of the novel 3-hydroxy **4d** and 3-chloro **4j** derivatives pyrroloisoquinoline compounds, which displayed excellent cytotoxic activity. The IC_{50} values of these compounds were determined and were found to compare satisfactorily with those of the commercially available drugs topotecan, irinotecan, etoposide, tamoxifen, and cisplatin.

Experimental

Chemistry

All reported melting points were measured in open capillaries using a Mel-Temp apparatus. ^{1}H-NMR spectra were recorded on a Avance III HD 700 MHz Brucker, Avance III HD 500 MHz Brucker, Avance 400 MHz Brucker, 300 MHz Jeol Eclipse, Fourier 300 MHz Brucker

spectrometers in deuterated chloroform ($CDCl_3$) solutions using TMS as the internal standard (δ = 0 ppm), ^{13}C-NMR spectra were recorded at 75, 100, 125, 150 and 175 MHz on the same instruments. The chemical shifts are reported in the δ scale in parts per million (ppm). The peak patterns are indicated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; brs, broad signal. The coupling constants (J) are reported in Hertz (Hz). IR spectra were obtained on a Magna-IR spectrometer. Mass spectra were recorded on Jeol JEM-AX505HA spectrometer by electronic impact (EI) detection at 70 eV for low-resolution and on a Jeol 5X102A mass spectrometer (Jeol Ltd.) with fast atom bombardment (FAB+) and electronic impact (EI) ionization detection for high-resolution measurements.

General procedure for synthesizing the pyrroles 2a'–2n

A solution containing the alkene (2.6 mmol) and 1-(1-isocyanoethylsulfonyl)-4-methylbenzene (Me-TosMIC) (0.6 g, 2.9 mmol) in Et_2O: DMSO (2:1, 15 mL) was added dropwise to a suspension of NaH (0.23 g, 5.7 mmol, 60% dispersion in mineral oil) in dry ether (5 mL). The mixture was stirred at room temperature for 1 h, then H_2O (15 mL) was added dropwise and the product was extracted with EtOAc (3x30 mL). The organic layer was washed with H_2O and brine (3x10 mL), dried with Na_2SO_4, and evaporated *in vacuo*. The residue was purified by flash column chromatography on silica gel to furnish the respective pyrrole. Physical and spectroscopic data of all compounds **2á-2n** are reported in supplementary material.

General procedure for the synthesis of the N-alkylpyrroles 3a'–3n

NaH (0.2 g, 5.7 mmol, 60% dispersion in mineral oil) was added portionwise to a solution of the corresponding pyrrole (2.3 mmol) and 2-bromo-4,5-dimethoxyphenethyl 4-methyl benzenesulfonate (1.9 g, 4.5 mmol) in dry dimethyl sulfoxide (10 mL). The mixture was

stirred at room temperature for 6 h, EtOAc (20 mL) was added, and the solution was washed with water and brine (3 x 10 mL). The organic layer was dried with Na_2SO_4 and evaporated *in vacuo*. The residue was purified by flash column chromatography on silica gel to furnish the respective *N* -alkylpyrrole. Physical and spectroscopic data of all compounds **3á-3n** are reported in supplementary material.

General procedure for synthesizing the 5,6-dihydropyrrolo[2,1-a]isoquinolines 4a', 4b, 4c, 4d', 4e, 4f, 4j, 4k', 4l', 4m, 4n.

To a refluxing solution of the *N*-alkylpyrrole in degassed dry toluene (10 mL), a solution of *n*-Bu_3SnH (1.0 mL, 3.7 mmol) in toluene (5 mL) was added dropwise (syringe pump) over 7 h. During that time, solid dilauroyl peroxide (DLP) was added portionwise (1.49 g, 3.7 mmol, 0.11 g/30 min). The solvent was removed under reduced pressure and the crude residue was purified by flash column chromatography on silica gel. Hexane was first added to remove the *n*-Bu_3SnBr, then hexane–EtOAc–Et_3N (70:25:5 to 50:45:5). Physical and spectroscopic data of all compounds **4á-4n** are reported in supplementary material.

Cell Culture and Assay for Cytotoxic Activity

Cell culture and assay for activity PC-3, U-251, K-562, HCT-15, MFC-7, and SKUL-1, were supplied by The National Cancer Institute(NCI), U.S.A. The cytotoxicity of tumors cells with the test compounds was determined using the protein-binding dye sulforhodamine B(SBR) in microculture assay to measure cell growth.[20] The cell lines were cultured in RPMI-1640 (Sigma Chemical Co., Ltd., St. Louis, MO, U.S.A.) supplemented with 10% fetal bovine serum which was purchased from Invitrogen Corporation, 2 mM L-glutamine, 10000 units/mL of penicillin G, 10000 µg/mL streptomycin and 0.25 μg/mL Fungizone(Gibco). They were maintained at 37^{o}C in a 5% CO_2 atmosphere with 95% humidity. For the assay,

5104 cell/mL (K-562, MCF-7), 7510 cell/ mL (U-251, PC-3) and 10104 cell/mL (SKLU-1, HCT-15), and 100 mL/well of these cells suspension was seeded in a 96-well microtiter plates and incubated to allow for cell attachment. After 24 h, 100 µL of each test compounds and positive substances were added to each well. Later 48 h, adherent cell cultures were fixed *in situ* by adding 50 mL od cold 50% (w/v) trichloroacetic acid (TCA) and incubated for 60 min at 4^{o}C. The supernatant was discarded and the plates were washed three times with water and air dried. Cultured fixed with TCA were stained for 30 min with 100 µL of 0.4% SRB solution. Protein-bounded dye was extraxted with 10mM unbuffered tris base and the optical densities were read on a Microplate Reader Synergy HT (Elx 808, BIO-TEK Instruments, Inc.), with a test wavelength of 515 nm. Results were expressed as IC_{50} values, they were calculated according to the protocol of Monks, were a dose-response curve was plotted for each compound, and the concentration giving 50% inhibition (IC_{50}) was estimated from non-linear regression equations. The IC_{50} value (mean standard error (S.E). [21]

Acknowledgments

This work was supported by Conacyt (Consejo Nacional de Ciencia y Tecnología), Project No. 131295 and Fellowship No. 223141. Financial support from the DGAPA, UNAM (project PAPIIT IN213407) is gratefully acknowledged. We also thank R. Patiño, N. Esturau, A. Peña, E. Huerta, B. Quiroz, and C. García, L. Velasco, J. Pérez, and V. Labastida (CIQ, UAEM) for technical support. During the experimentation process Rosa María Chávez Santos was a Conacyt Graduate Scholarship holder (No. 223441).

Conflict of Interest The authors declare no conflict of interest

Supplementary Materials The online version of this article contains supplementary materials

References

1) Ferlay J., Soerjomataram I., Dikshit R., Eser S, Mathers C., Rebelo M., Parkin D. M., Forman D., Bray F., *Int. J. Cancer*., **136**, E359–E386 (2015).

2) Fitzmaurice C., *JAMA Oncol.,* **1**, 505-527 (2015).

3) Reyes-Gutiérrez P. E., Camacho J. R., Ramírez-Apan M. T., Osornio Y. M., Martínez R., *Org. Biomol. Chem*., **8**, 4374-4382 (2010). Bauser M., Ergüden J. K., Flubacher D., Naab P., Repp T. H., Stoltefuss J., Burkhardt N., Sewing A., Schauer M., Schlemmer K. H., Weber O., Boyer S. J., Miglarese M., Ying S., Niewöhner U., (2003) 3-substituted pyrrolo[2.1-*a*]isoquinoline derivatives. WO2003014 117A1, PCT/US2002/024877., http://google.com/patents/WO2003014117A1?cl=en

4) Sano S., Matsumoto T., Nanataki H., Tempaku S., Nakao M., *Tetrahedron Lett.,* **55**, 6248-6251 (2014).

5) Heck R., *Metals Rev.,* **24**, 58-63 (1980).

6) Valeur E., Bradley M., *Chem. Soc. Rev.,* **38**, 606-631 (2009).

7) Van Leusen A. M., Siderius H., Hoogenboom B. E., Leusen D., *Tetrahedron Lett.,* **52**, 5337-5340 (1972).

8) Guerrero M. A., Cruz-Almanza R., Miranda L. D., *Tetrahedron.,* **59**, 4953-4958 (2003).

9) Amatore C., Jutand A., *Acc. Chem. Res*., **33**, 314-321 (2000).

10) Xia Y. M., Xia J., Chai C., *Chem. Pap*., **68**, 384-391 (2014).

11) Horita K., Yoshioka T., Tanaka T., Oikawa Y., Yonemitsu O., *Tetrahedron.,* **42**, 3021-3028 (1986).

12) Nakhi A., Rahman M. S., Archana S., Kishore R., Seerapu G. P. K., Kumar K. L., Haldar D., Pal M., *Bioorg. Med. Chem. Lett.,* **23**, 4195-4205 (2013).

13) Sajiko H., Kuno H., Hirota K., *Tetrahedron Lett.,* **38**, 399-402 (1997).

14) Moreira-Lima L., Barreiro E. J., *Curr. Med. Chem.,* **12**, 23-49 (2005).

15) Khalife R., Hodroj M., Fakhoury R., Rizk S., *Planta Med*., **82**, 312-321 (2016).

16) Phelps M. A., Sparreboom A., **32**, 2287-2289 (2014).

17) Pommier Y., Leo E., Zhang H. L., Marchand C., *Chem. Biol.,* **17**, 421–433 (2010).

18) Hackshaw A., Roughton M., Forsyth S., Monson K., Reczko K., Sainsbury R., Baum M., *J. Clin. Oncol.,* **29**, 1657-1663 (2011).

19) Florea A. M., Büsselberg D., *Cancers*., **3**, 1351-1371 (2011).

20) Musser J. H., Kreft A., *J. Med. Chem.,* **35**, 2501-2524 (1992).

21) Monks A., Scudiero D., Skehan P., Schoemaker R., Paull K., Vistica D., Hose C., Langley J., Cronise P., Vaigro-Wolff A., Gray-Goodrich M., Campbell H., Mayo J., Boyd M., *J. Natl. Cancer Inst.,* **83**,757-766 (1991).

FIGURES

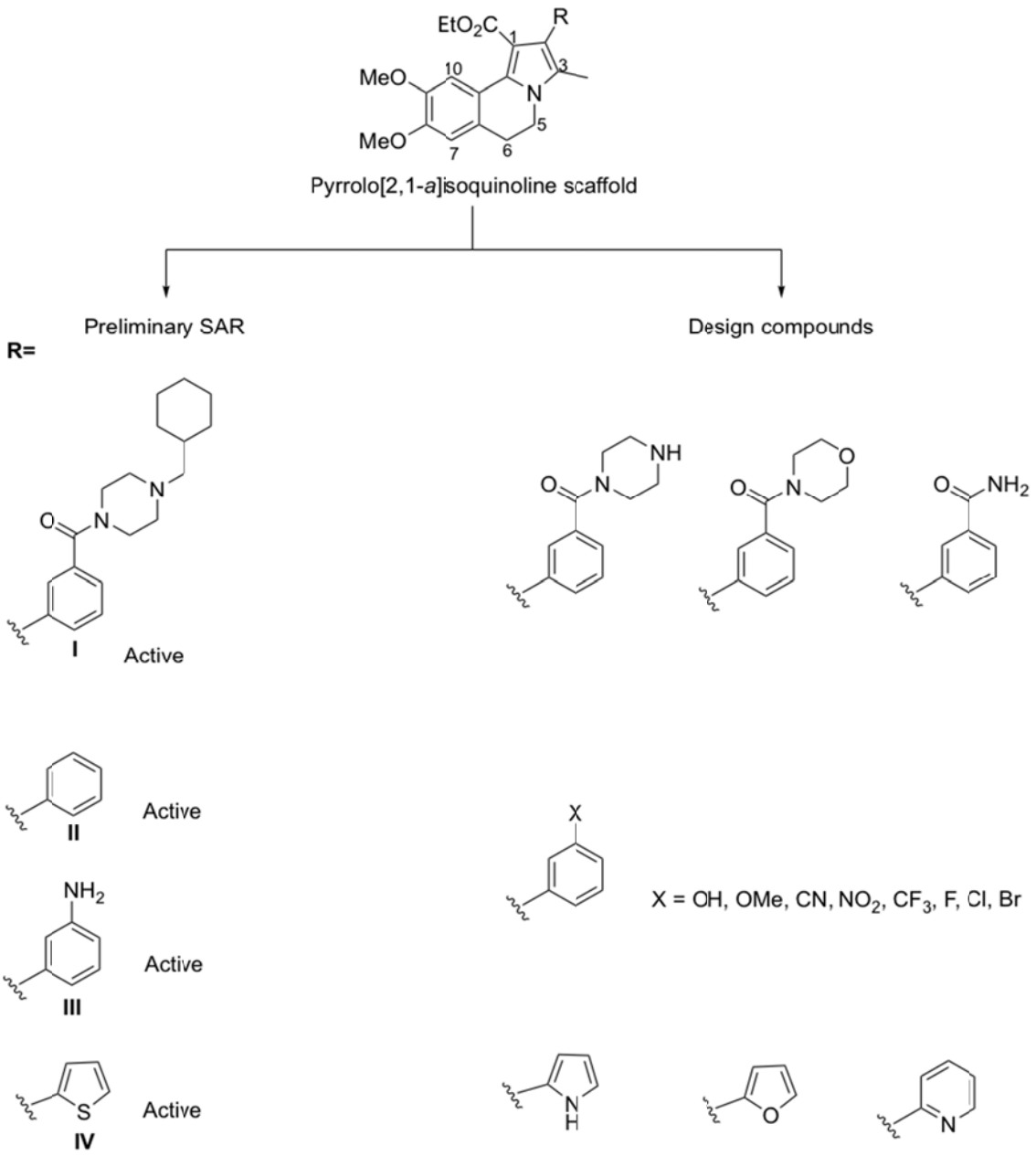


Figure 1. Preliminary SAR of pyrroloisoquinolines **I-IV** and designed compounds

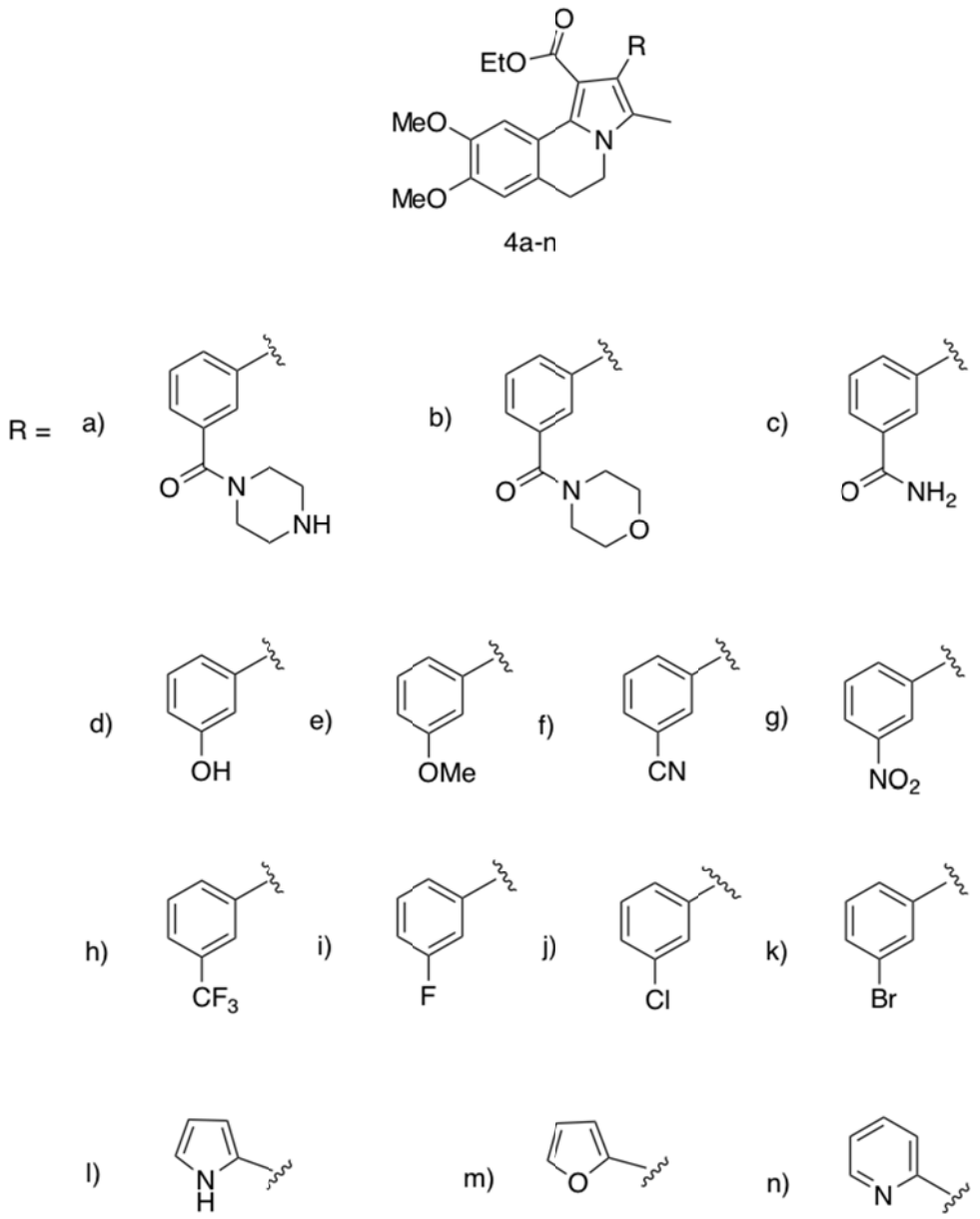


Figure 2. Proposed compounds to be synthesized and evaluated to determine the activity effects of the structural modifications relative to the activities of structures **I–IV.**

CHARTS

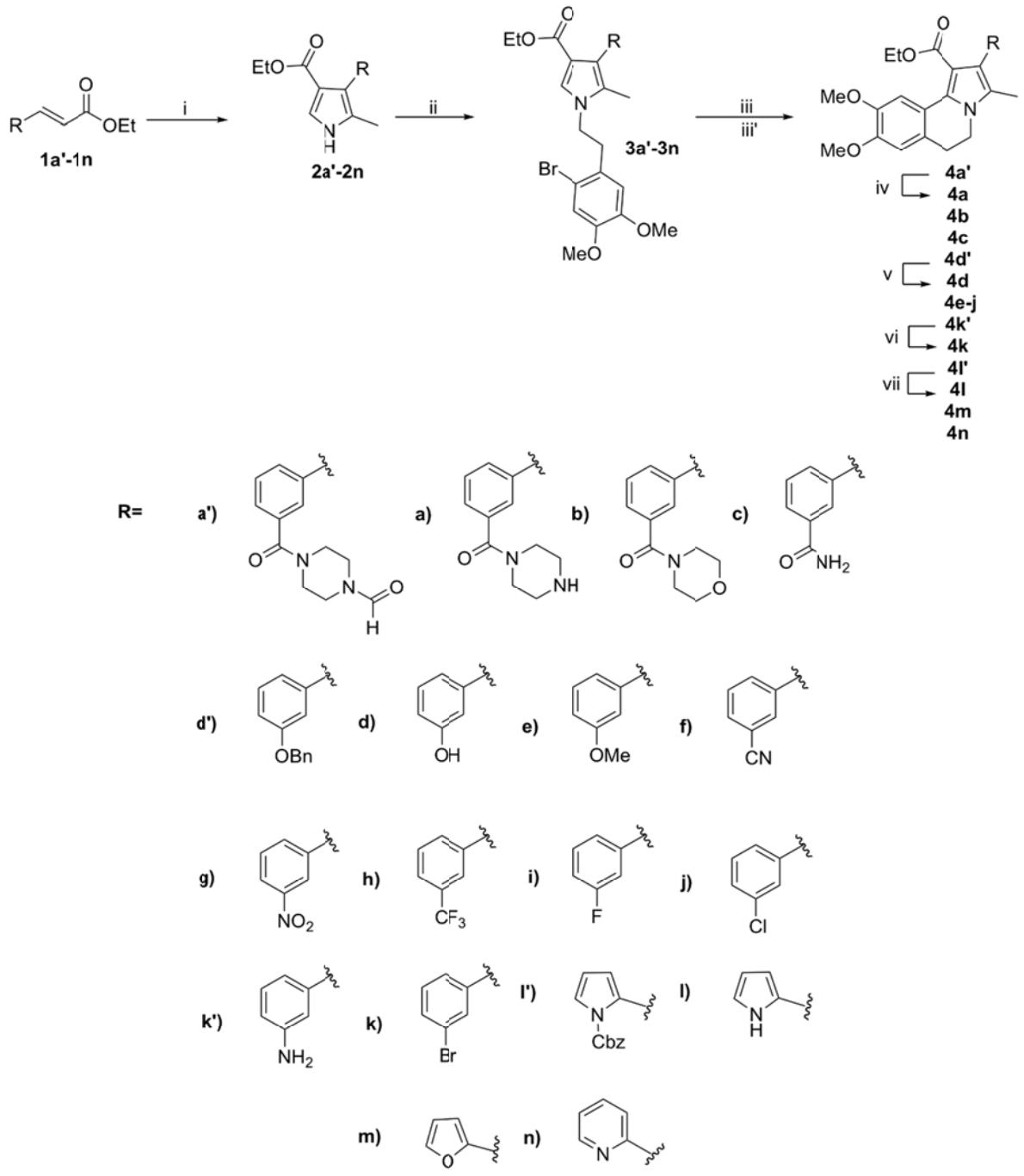


Chart 1. Synthetic route of compounds **4a-n**. Reagents and conditions: (i) NaH, CH_3-TosMIC, diethyl ether: DMSO (2:1), 0 °C to RT.; (ii) NaH, DMSO, 2-bromo-4,5-dimethoxyphenethyl 4-methylbenzenesulfonate, RT; (iii) *n*-Bu_3SnH, DLP, Toluene, reflux; (iii') $Pd(OAc)_2$, PPh_3, Et_3N, CH_3CN, reflux; (iv) Hydrazine/EtOH, 60 °C; (v, vii) H_2, Pd/C EtOH, RT; (vi) *t*-BuONO, $CuBr_2$, CH_3CN, 0 °C.

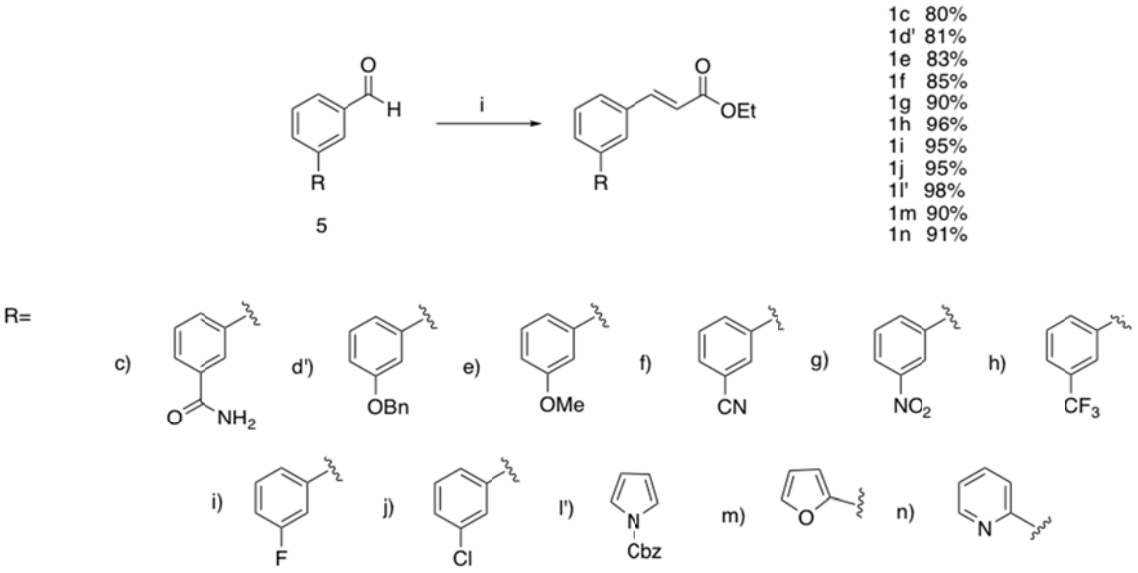


Chart 2. Synthetic route to the starting **c**ompounds **1c, 1d'**, **1e–j**, **1l'**, **1m**, and **1n**. Reagents and conditions: (i) ethyl 2-(die**t**hoxyphosphoryl)acetate, NaH, DMF –60°C

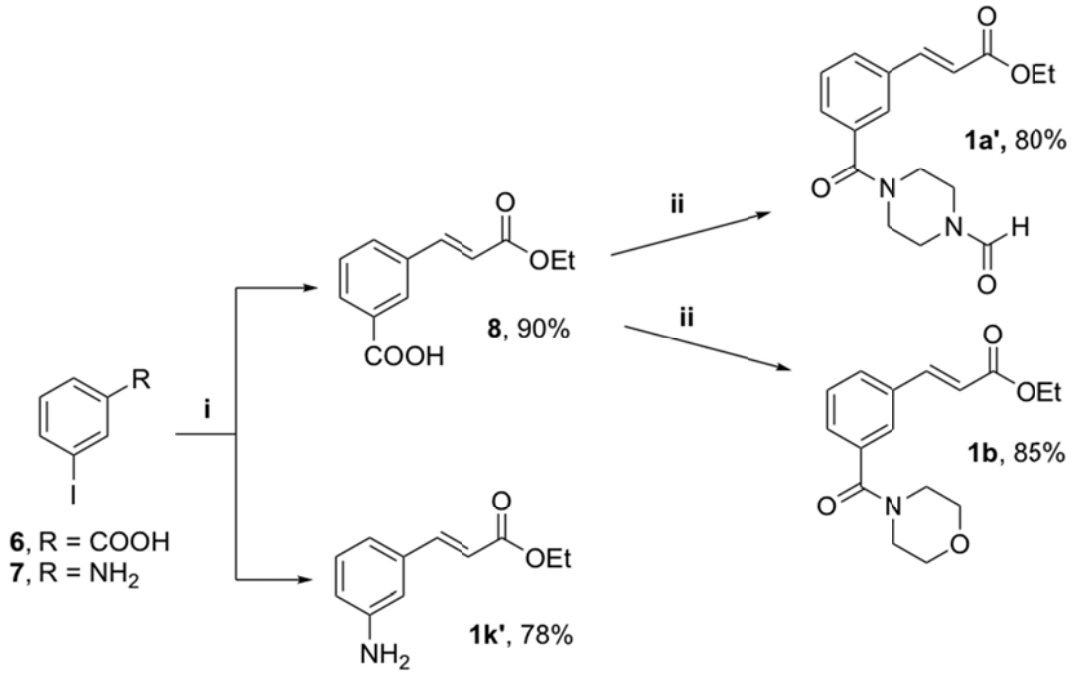
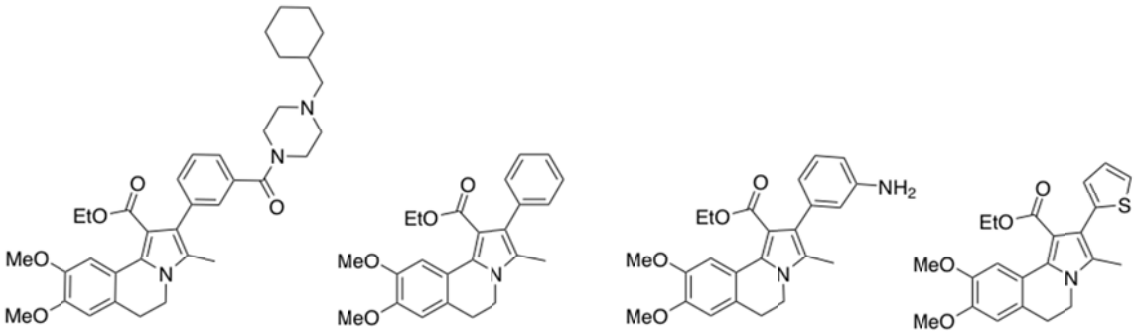


Chart 3. Synthetic route to compounds **1a', 1b** and **1k'**. Reagents and conditions: (i) ethyl acrylate, $Pd(OAc)_2$, PPh_3, Et_3N, CH_3CN, reflux; (ii) DCC, DCM, amine, RT.

TABLES

Table 1. The IC_{50} values (mM) of compounds **I** to **IV** in the six cancer cell lines.[a]

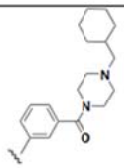
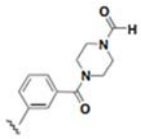
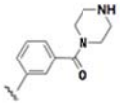
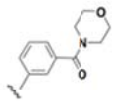
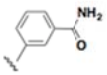


I II III IV

Compd.	PC-3	U-251	K-562	HCT-15	MCF-7	SKLU-1
I	0.16±0.01	0.05±0.00	0.16±0.01	0.02±0.01	5.58±0.04	0.02±0.00
II	18.15±0.60	4.86±0.60	76.78±7.30	0.14±0.06	25.20±2.00	0.59±0.00
III	21.20±1.20	5.96±0.50	2.50±0.80	0.01±0.00	1.30±0.10	0.10±0.01
IV	8.47±0.23	6.99±0.67	4.07±0.49	0.59±0.05	7.41±0.09	2.13±0.03

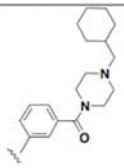
[a] Results are expressed as IC_{50} values in units of μM ± standard error. The values indicate the mean calculated from experiments conducted in triplicate

Table 2 Effects of modifying the cyclohexylmethylpiperazinyl group on the antiproliferative activity[a]

Compd.	R=	PC-3	U-251	K-562	HCT-15	MCF-7	SKLU-1
I		**0.16±0.01**	**0.05±0.00**	**0.16±0.01**	**0.02±0.01**	5.58±0.04	**0.02±0.001**
4a'		10.20±1.40	11.10±0.70	8.12±0.70	11.40±0.70	8.32±0.90	7.98±0.40
4a		6.39±0.50	6.89±0.80	3.45±0.20	5.23±0.50	7.26±0.60	6.28±0.70
4b		N.A	N.A	N.A	N.A	N.A	N.A
4c		1.23±0.10	1.42±0.10	1.07±0.08	1.34±0.30	**1.09±0.10**	1.13±0.10

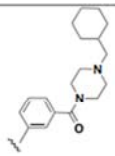
[a] Results are expressed as IC_{50} values in units of μM ± standard error. The values indicate the mean calculated from experiments conducted in triplicate. N.A.: this compound was not active. The bold numbers represent the highest activities of the compounds tested.

Table 3 Effects of *meta*-substituent on 3-phenyl moiety on the antiproliferative activity and comparison with the activities of commercially available drugs [a]

Compd.	R=	PC-3	U-251	K-562	HCT-15	MCF-7	SKLU-1
I		0.16±0.01	0.05±0.00	0.16±0.01	0.02±0.01	5.58±0.04	0.02±0.001
II		18.15±0.60	4.86±0.60	76.78±7.30	0.14±0.06	25.20±2.00	0.59±0.00
III		21.20±1.20	5.96±0.50	2.50±0.80	**0.01±0.00**	1.30±0.10	0.10±0.00
4d		**0.76±0.50**	6.12±0.40	5.47±0.70	0.01±0.00	5.72±0.40	**0.05±0.01**
4e		3.26±0.10	3.31±0.40	2.65±0.10	0.69±0.05	2.35±0.20	0.77±0.08
4f		3.86±0.10	3.17±0.30	1.98±0.05	2.53±0.20	4.08±0.20	2.93±0.08
4g		8.26±1.00	12.66±0.50	8.28±1.10	0.10±0.04	13.98±0.70	1.26±0.30
4h		2.30±0.20	2.50±0.40	3.30±0.20	1.90±0.20	3.10±0.30	1.70±0.10
4i		2.20±0.09	3.10±0.20	1.30±0.20	2.50±0.30	1.70±0.07	3.30±0.10
4j		0.91±0.01	**0.37±0.04**	**0.33±0.03**	0.25±0.02	**0.88±0.09**	0.76±0.07
4k		22.90±0.90	23.60±0.90	5.20±1.20	5.60±0.20	57.70±1.00	3.60±0.60
Topotecan					**0.50±0.05**	**0.10±0.02**	**2.00±0.10**
Irinotecan					**33.09±3.40**		**34.62±2.30**
Etoposide			**1.70±0.30**	**11.30±2.50**			**4.10±0.60**
Tamoxifen						**12.80±1.10**	
Cisplatin		**8.30±0.70**	**3.30±0.60**				

[a] Results are expressed as IC_{50} values in units of μM ± standard error. The values indicate the mean calculated from experiments conducted in triplicate. The bold numbers represent the highest activities of the compounds tested.

Table 4. Effects of aromaticity of C-2 substituent on the antiproliferative activity[a]

Compd.	R=	PC-3	U-251	K-562	HCT-15	MCF-7	SKLU-1
I		0.16±0.01	0.05±0.00	0.16±0.01	0.02±0.01	5.58±0.04	0.02±0.00
II		18.15±0.60	**4.86±0.60**	76.78±7.30	**0.14±0.06**	25.20±2.00	**0.59±0.00**
IV		8.47±0.23	6.99±0.67	**4.07±0.49**	0.59±0.05	**7.41±0.09**	2.13±0.03
4l		10.50±0.24	8.99±0.68	6.50±0.67	0.90±0.06	13.60±0.60	5.60±0.05
4m		**2.97±0.10**	8.67±0.10	8.54±0.50	1.72±0.20	14.70±1.00	6.76±0.09
4n		21.70±0.20	24.70±1.10	9.10±0.90	14.00±1.40	23.70±1.00	15.60±1.20

[a] Results are expressed as IC_{50} values in units of μM ± standard error. The values indicate the mean calculated from experiments conducted in triplicate. The bold numbers represent the highest activities of the compounds tested.