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Syntheses and antiproliferative evaluation of oxyphenisatin derivatives

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Abstract—Syntheses and structure–antiproliferative relationship for oxyphenisatin analogues are described. The cell proliferation data showed that the presence of substituents (especially F, Cl, Me, CF_3, and OMe) in the 6- and 7-position of oxyphenisatin markedly enhanced the potency in the MDA-468 cell line without affecting the MDA-231 cell line. The best compounds from this series showed low nanomolar antiproliferative activity towards the MDA-468 cell line and a 1000-fold selectivity over the MDA-231 cell line.

Cancer is the second leading cause of death in developed countries, accounting for nearly one in five deaths. One characteristic of cancer cells is their highly proliferative nature. Consequently, inhibition of proliferative pathways is considered an effective strategy to fight cancer. One way to identify compounds with antiproliferative effects is to screen for compounds which reduce proliferation in one cancer cell line and counter screen against another cancer cell line. Important advantages of this approach are that the active compounds are reasonably cell permeable and water soluble. Furthermore, compounds with nonspecific toxicity are eliminated and, as the functional end-effect is measured in whole cells, the risk of observing compensatory mechanisms at a later stage is significantly reduced. However, in contrast to screening against an isolated molecular cancer target this approach requires further studies to elucidate the exact mode of action.

In an anticancer project we identified 6-chloro-3,3-bis-(4′-hydroxyphenyl)-7-methyl-1,3-dihydroindole-2-one **1** (see Fig. 1) as a potent inhibitor of the cell proliferation in the MDA-468 cell line (IC_{50} = 20 nM). Furthermore, the compound was more than 100-fold less active towards the MDA-231 cell line (IC_{50} > 3 μM). This screening approach was designed to identify compounds with a cell selectivity profile similar to CCI-779, a novel mTOR inhibitor.[1] Natarajan et al. recently published cell growth inhibition data on a similar series of which the lead compound is the unresolved mixture of chiral compound **2**.[2] They compared the activity of these compounds to that of clotrimazole **3** and suggested the

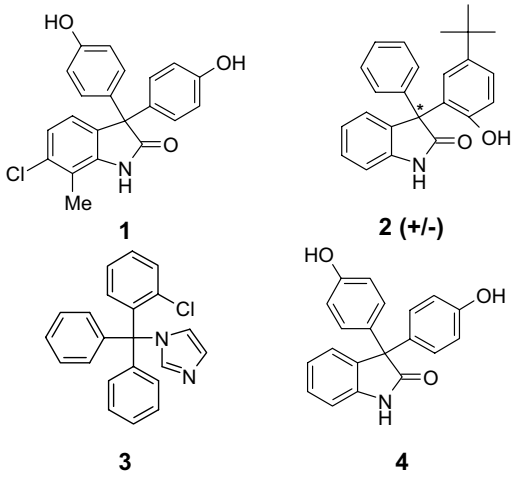

Figure 1. Structure of compound **1**, Lead compound of reference 1b **2**, Clotrimazole **3**, and Oxyphenisatin **4**.

Keywords: Antiproliferative; Anticancer; MDA-468 cell line; Phenisatin; Whole cell assay.
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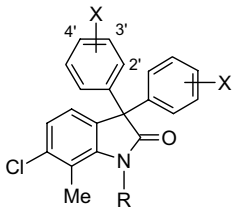
Table 1. Role of the hydrogen donor groups of compound **1**



Compound	MDA-468 IC_{50}[a] (μM)	MDA-231 IC_{50}[a] (μM)	X	R
4 (oxyphenisatin)	0.112	na	—	—
1	0.020	na	4′-OH	H
1a	na	na	4′-OH	Me
1b	0.238	na	4′-H	H
1c	0.291	na	4′-F	H
1d	1.8	na	4′-OMe	H
1e	2.6	na	4′-NH_2	H
1f	na	na	4′-NMe_2	H
1g	na	na	4′-NHAc	H
1h	na	na	4′-$NHSO_2Me$	H
1i	na	na	2′-OH	H

[a] Values are means of two experiments (na, not active (>3 μM)).

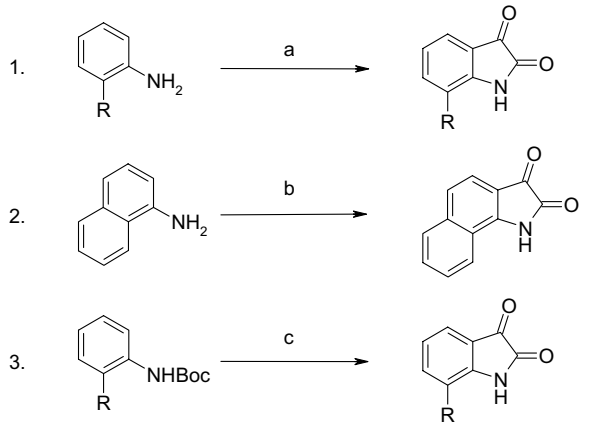
antiproliferative effect to be mediated by translation initiation inhibition.

However, in our two cell lines, clotrimazole **3** showed no selectivity and an IC_{50} of more than 10 μM in both assays. Since compound **1** is a substituted oxyphenisatin (**4**), the latter was included in the screen (see Fig. 1). The cell proliferation data showed oxyphenisatin **4** to be equally selective, but ~5-fold less potent (see Table 1). Oxyphenisatin acetate is a laxative which was used for more than 40 years as a non-prescription drug before it was taken off the market in 1972 due to hepatotoxicity.[3] The hepatic reaction to oxyphenisatin is, however, presumably due to a hypersensitivity response rather than a nonspecific toxic effect.[4]

In the present work, we describe the syntheses and structure–activity relationship of these symmetrical oxyphenisatin derivatives using antiproliferative data from the MDA-468 cell line. We specifically excluded chiral molecules to avoid potential resolution problems during further development phases.

Isatins are commonly used intermediates for accessing oxyphenisatins and several procedures exist in the literature to form this particular ring system.[5] In this study, we have primarily used the synthetic methods shown in Scheme 1. The Sandmeyer methodology[6] (see example 1 in Scheme 1) is the oldest and most frequently used synthetic approach towards isatins. The key step involves the cyclisation of the intermediate obtained by reacting anilines, chloral hydrate and hydroxylamine.

In the case of meta-substituted anilines, the cyclisation step in the Sandmeyer procedure can potentially afford



Scheme 1. Reagents and conditions: (a) CCl_3CHO, H_2NOH, 60 °C then H_2SO_4, 90 °C; (b) AcOH, diethyl ketomalonate, then KOH(aq); (c) *t*BuLi, diethyl oxalate, then 180 °C, <5 mmHg.

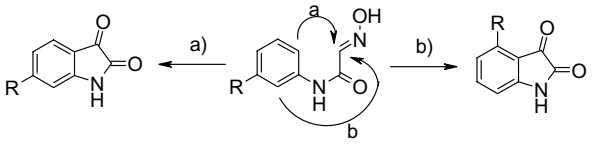
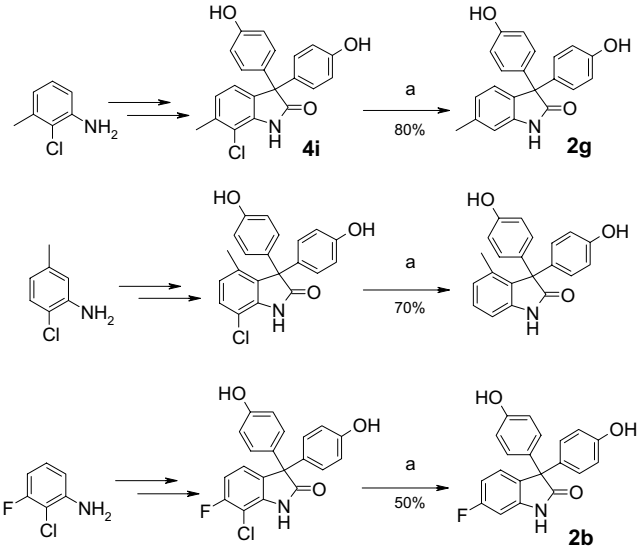


Figure 2.

a mixture of two isomers (see pathway a or pathway b in Fig. 2).[7]

We controlled the regiochemistry of the product formation by use of a chlorine atom to block one of two potential cyclisation positions and then subsequently removed the halogen by a catalytic dehalogenation with a $Pd/C/H_2$ system affording either the 4 or the 6-substituted oxyphenisatin (several examples are shown in Scheme 2).[8]



Scheme 2. Reagents and condition: (a) Pd/C, H_2, AcOK, rt, MeOH.

The Martinet isatin procedure[9] (see example 2 in Scheme 1) involves the reaction of diethyl keto-malonate hydrate with anilines in the presence of acid, followed by the oxidative decarboxylation of the resulting intermediate.

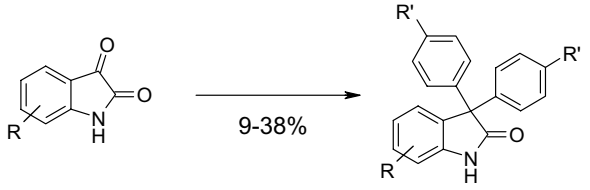
The ortho-lithiation procedure[10] (see example 3 in Scheme 1) proceeds by an α-directed deprotonation of *N*-Boc protected aniline, followed by trapping of the aryllithium intermediate with diethyl oxalate, and cyclisation under electrophilic conditions results in the isatin formation.

We have obtained the desired oxyphenisatin derivatives, quickly and in large numbers via a double Friedel–Crafts reaction[11,12] on the keto function of the isatins using substituted phenyl derivatives containing a combination of ortho- or para-orientating groups (see Scheme 3).

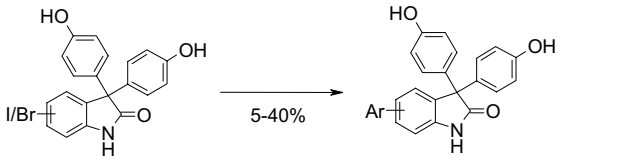
Further functionalisations were also performed on the prepared oxyphenisatins. For example Suzuki reactions[13] were carried out on 5/7-bromo and 5/7-iodo-3,3-bis-(4-hydroxy-phenyl)-1,3-dihydro-indol-2-one with boronic acid and palladium tetrakis triphenyl phosphine, better results were obtained from the bromo precursors (see Scheme 4).

Finally, a Sonogashira coupling [14] of derivative **3d** with TMS acetylene, followed by TBAF treatment, afforded compound **3h** in a low yield of 5% (see Table 3).

To explore the structural–antiproliferative activity relationship of compound **1**, we performed a systematic investigation of the functionalities present. Initially we evaluated the importance of the hydrogen bond donor moieties.



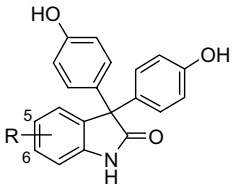
Scheme 3. Reagents and condition: Ph-R′, AcOH, H_2SO_4 or TfOH, 90 °C.



Ar = 5-phenyl	**2j**	Ar = 7-phenyl	**3m**
Ar = 5-(2-thienyl)	**2k**	Ar = 7-(2-thienyl)	**3n**
Ar = 5-(4-pyridyl)	**2l**	Ar = 7-(4-pyridyl)	**3o**

Scheme 4. Reagents and condition: $Pd(PPh_3)_4$, Ar–$B(OH)_2$, K_2CO_3, DME, 80 °C.

Table 2. Substituents in the 5- and 6-position of oxyphenisatin

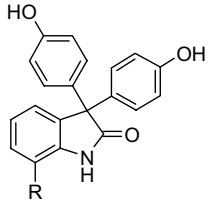


Compound	MDA-468 IC_{50}[a] (μM)	MDA-231 IC_{50}[a] (μM)	R
2a	0.170	na	5-F
2b	0.028	na	6-F
2c	0.152	na	5-Cl
2d	0.190	na	5-Br
2e	0.208	na	5-I
2f	0.183	na	5-Me
2g	0.077	na	6-Me
2h	0.173	na	5-OMe
2i	0.191	na	6-OMe
2j	na	na	5-Ph
2k	na	na	5-(2-Thienyl)
2l	na	na	5-(4-Pyridyl)
2m	na	na	COOH

[a] Values are means of two experiments (na, not active (>3μM)).

From Table 1 it is evident that the OH-functions attached to the 4-position of the phenyl ring systems are crucial as removing or methylating the OH function (**1b** and **1d**), moving the OH-function to the 2-position (**1i**) or replacing the 4-OH-function with phenol bioisosters in the same position (**1e**, **1g–h**) all resulted in compounds with a significantly reduced potency.

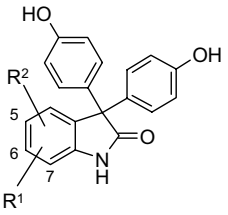
Table 3. Substituents in the 7-position of oxyphenisatin



Compound	MDA-468 IC_{50}[a] (μM)	MDA-231 IC_{50}[a] (μM)	R
3a	0.034	na	F
3b	0.006	na	Cl
3c	0.006	na	Br
3d	0.062	na	I
3e	0.027	na	Me
3f	0.004	na	CF_3
3g	0.057	na	CN
3h	0.090	na	C≡CH
3i	0.262	na	OMe
3j	0.330	na	Et
3k	na	na	*i*-Pr
3l	na	na	*t*-Bu
3m	na	na	Ph
3n	na	na	2-Thienyl
3o	na	na	4-Pyridyl
3p	na	na	COOH
3q	na	na	$CONMe_2$

[a] Values are means of two experiments (na, not active (>3 μM)).

Table 4. Disubstituted oxyphenisatin derivatives



Compound	MDA-468 IC_{50}[a] (μM)	MDA-231 IC_{50}[a] (μM)	R^1	R^2
4a	0.037	na	5-F	7-F
4b	0.100	na	5-F	7-Me
4c	0.162	na	5-Me	7-Me
4d	0.160	na	5-OMe	7-Me
4e	0.003	na	6-F	7-F
4f	0.006	na	6-F	7-Me
4g	0.089	na	6-Br	7-Me
4h	0.007	na	6-Me	7-Me
4i	0.006	na	6-Me	7-Cl
4j	0.009	na	6-OMe	7-Me
4k	0.021	na	6-Cl	7-F
4l	0.055	na	Cyclo-	Pentyl
4m	na	na	Cyclo-	Hexyl

[a] Values are means of two experiments (na, not active (>3μM)).

Interestingly, methylating the amide function (**1a**) also resulted in complete loss of antiproliferative activity.

We then explored the structure antiproliferative relationship of monosubstituted oxyphenisatin derivatives carrying different substituents in either the 5-, 6- or 7-position (see Tables 2 and 3). The oxyphenisatins with a substituent at the 4-position did not show any relevant activity.

The data indicate that halogens and small lipophilic substituents in the 6- and 7-position (**2b**, **2g**, **3a**, **3b**, and **3f**) are important for the antiproliferative activity. Whereas, larger substituents (**3k**, **3l**, and **3m**) or polar substituents (**2m**, **3i**, **3p** and **3q**) reduce the potency.

Finally, we evaluated disubstituted oxyphenisatin derivatives carrying two small and lipophilic substituents. Table 4 summarizes the results and confirms the importance of having relatively small and lipophilic substituents in the 6- and/or 7-position.

The two lead compounds (**3f** and **4e**) were further profiled and both showed acceptable Caco-2 permeability (19.6 and 43.1 nm/s, respectively) and aqueous solubility (0.64 and 0.23 mg/mL, respectively, at pH 7.0). They are both highly bound to serum protein (99% and 98%, respectively), but show no evidence of P-gp mediated efflux using Vinblastine as internal control and Verapamil as P-gp inhibitor. Both compounds showed poor inhibition (>10 μM) of CYP1A2, 2D6 and 3A4, but moderate inhibition (1–2 μM) of CYP2C9 and 2C19 probably due to the presence of the phenol moieties.[15]

In conclusion, we have used a cell-based screening approach to assess structure–activity relationships and identified two low nanomolar compounds with reasonable physicochemical and in vitro ADME profiles. The in vivo activity of this compound series has also been confirmed in appropriate xenograft models.[12]

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