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Synthesis, biological evaluation and structure–activity relationship studies of isoflavene based Mannich bases with potent anti-cancer activity

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ABSTRACT

Phenoxodiol, an analogue of the isoflavone natural product daidzein, is a potent anti-cancer agent that has been investigated for the treatment of hormone dependent cancers. This molecular scaffold was reacted with different primary amines and secondary amines under different Mannich conditions to yield either benzoxazine or aminomethyl substituted analogues. These processes enabled the generation of a diverse range of analogues that were required for structure–activity relationship (SAR) studies. The resulting Mannich bases exhibited prominent anti-proliferative effects against SHEP neuroblastoma and MDA-MB-231 breast adenocarcinoma cell lines. Further cytotoxicity studies against MRC-5 normal lung fibroblast cells showed that the isoflavene analogues were selective towards cancer cells.

Hormone-dependent cancers such as breast and prostate cancers are on the increase in most Western countries. One in eight Australian women will be diagnosed with breast cancer by the time they turn 85. The risk of breast cancer in women increases with age, with the majority of women diagnosed being between 50 and 69 years of age. In 2013, approximately 15,000 women were diagnosed with breast cancer and 2700 women lost their lives to breast cancer.[1–4] Prostate cancer is a common cancer in men, accounting for 32.4% of all male cancers in Australia.[2–5] The risk of developing prostate cancer increases with age, with 85% of cases diagnosed in men over 65 years of age. In 2012, prostate cancer was estimated to account for 15% of the total burden of cancer in men in Australia, second only to lung cancer.[2–5]

Phytoestrogens are estrogen-like substances found in plants such as beans, grains, vegetables, fruit and seeds. The high dietary intake of phytoestrogens in Asian populations, due in part to their high soy food consumption, has been linked to lower incidences of hormone-dependent cancers such as prostate and breast cancer.[5] Among the various classes of phytoestrogens, isoflavones have been among the most widely studied due to their marked estrogenic activities.[6] Phenoxodiol **1**, a synthetic isoflavene derivative, has been tested in clinical trials for the treatment of drug-resistant ovarian (NCT00382811) and prostate (NCT00557037) cancer. Its broad spectrum anti-cancer activity has been attributed to its ability to induce mitotic arrest, terminal differentiation and apoptosis in cancer cells.[7]

Phenols undergo the Mannich reaction quite readily, and the resultant product is typically *ortho* substituted relative to the hydroxyl group. The Mannich reactions of isoflavones containing a hydroxyl group at C7 give products substituted at either the C6 or C8 position, as the pendant ring B is less activated and does not compete with ring A under these reaction conditions. However, for phenoxodiol **1**, the combination of the electron-donating hydroxyl group on C7, the activating *para*-ether oxygen and the absence of a C4 ketone group make the C6 position of phenoxodiol **1** more reactive than the C8 position.

Khilya and co-workers generated the 6,8-bis-piperdin-1-yl-methyl- and 6,8-bis-piperazin-1-ylmethyl-substituted analogues of isoflavones, biochanin A and orobol through Mannich bases.[8] However, the synthesis of Mannich bases of isoflavenes has not been investigated. Recently, Wang et al. chose isoflavones as the

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main scaffold to synthesize C8 substituted Mannich bases and oxazinyl isoflavonoid compounds where the oxazinyl ring is fused at C8 and C7 hydroxyl group via the Mannich reaction with good in vitro anti-tumour activities.[9] These studies have shown that isoflavones readily undergo the Mannich reaction and are able to form the corresponding Mannich bases with high chemo- and regioselectivities. Due to its potent anti-proliferative activities, and in continuation of our extensive research into isoflavenes,[10–12] it is of interest to synthesise new aminomethyl and oxazinyl fused analogues of phenoxodiol and evaluate their anti-cancer properties.[13,14]

Initial attempts towards the Mannich reaction involved treating the isoflavene phenoxodiol **1** with different primary amines. Phenoxodiol **1** was reacted with formaldehyde and primary amines in a molar ratio of (1:12:2) in EtOH.[9] The progress of the reaction was monitored by TLC and the crude products were recrystallized from DCM and *n*-hexane. Generally, the reaction proceeded smoothly in all cases with aminomethylation occurring at the C6 position followed by benzoxazine formation (Scheme 1).

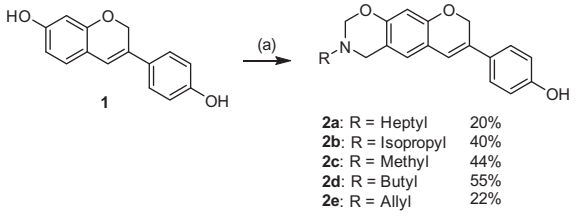
Interestingly, when this reaction was carried out with benzylamine, NMR analysis of the reaction product revealed a different structure than expected. This was determined to be the di-substituted benzoxazine **2f** (Scheme 2).

In order to synthesize a mono-substituted benzylamine benzoxazine product **2g**, the reaction was attempted at room temperature and monitored via TLC. It was observed that the reaction needed a longer time period of 48 h and that the mono-benzoxazine product **2g** precipitated cleanly from the reaction mixture and was collected by filtration in 70% yield and no recrystallization was needed. This indicates that the duration of the reaction and the temperature had a significant effect on the type of product formed and the yield. This further highlights the different reactivity of the phenoxodiol **1** to Mannich reactions at different temperatures. Based on these findings, further reactions with primary amines were carried out under these conditions to give the various desired analogues in 54–82% yield (Scheme 3).

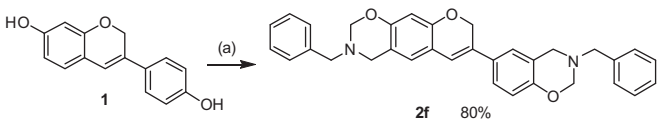
When the ratio of the phenoxodiol **1**, formaldehyde and benzylamine changed from 1:12:6 to 1:12:2, aminomethyl compound **3a** was formed instead of the expected benzoxazine product **2g** (Scheme 4). It was hypothesized that the excess of benzylamine led to complete consumption of formaldehyde, preventing further reaction to the benzoxazine. As a result, it was clear that by varying the molar ratio of the reactants the formation of the products could be controlled. Furthermore, literature reports of Mannich reactions confirm that the structure of the reaction product, whether a benzoxazine or aminomethyl species, depends on the ratio of the reagents used.[15–17]

Subsequently, a series of primary amines was reacted with lower concentration of formaldehyde to form aminomethyl-substituted phenoxodiols **3b–f** (Scheme 5). Briefly, the formation of **3f** was achieved by reacting phenoxodiol **1** with 37% aqueous formaldehyde and ethanolamine in a molar ratio of 1:5:4 in EtOH which proceeded smoothly with aminomethylation occurring at the C6 position to give **3f** in 55% yield (Scheme 5).

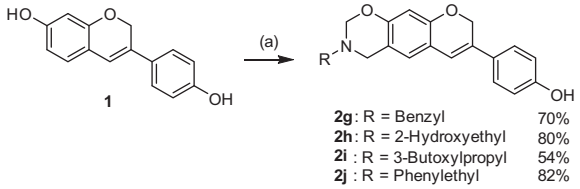
The low yield of the isopropylamine-derived compound **3b** was attributed to the steric bulk of isopropylamine and the formation



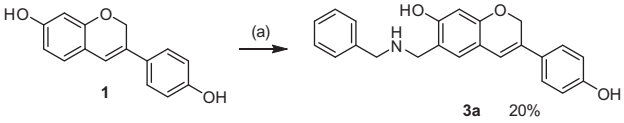
Scheme 1. Reagents and conditions: (a) primary amines (2 equiv), formaldehyde (12 equiv), EtOH, 24 h, reflux.



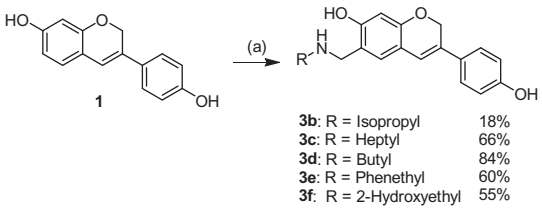
Scheme 2. Reagents and conditions: (a) benzylamine (2 equiv), formaldehyde (12 equiv), EtOH, 24 h, reflux.



Scheme 3. Reagents and conditions: (a) primary amines (2 equiv), formaldehyde (12 equiv), EtOH, 48 h, rt.



Scheme 4. Reagents and conditions: (a) benzylamine (6 equiv), formaldehyde (12 equiv), EtOH, 48 h, rt.



Scheme 5. Reagents and conditions: (a) primary amines (4 equiv), formaldehyde (5 equiv), EtOH, 48 h, rt.

of the benzoxazine side product. In order to improve the yield for the isopropylamine reaction, parameters such as temperature and solvent were investigated. Initially the reaction was carried out in 1,4-dioxane at room temperature, but with very little product formation. Then, the reaction was heated to 70 °C and after 48 h, the aminomethyl-substituted compound precipitated cleanly from the reaction mixture, but in poor yield. However, TLC analysis of the filtrate showed that some benzoxazine had also formed in the reaction. It was evident that the molar ratio of the formaldehyde was difficult to control and the formaldehyde left after the generation of the iminium ions reacted with the aminomethyl-substituted compound to form the benzoxazine.

To overcome this problem, other methods were also investigated for the synthesis of aminomethyl-substituted isoflavenes. One possibility was to use a formyl-substituted analogue of phenoxodiol **1**, which under reductive amination conditions could yield the desired aminomethyl-substituted phenoxodiol.

Hofslokken and Skattebol have reported a method for preparation of *ortho* formyl derivatives of phenols, which could be applied to the phenolic phenoxodiol.[18] Following the literature method, paraformaldehyde was added into the mixture of phenoxodiol **1**, anhydrous magnesium dichloride and triethylamine in the ratio 3:1:2:2 in THF. The reaction was heated at reflux for 4 h. Then the crude product was washed with 5% aq HCl to yield a C6-formyl phenoxodiol **4** (Scheme 6).

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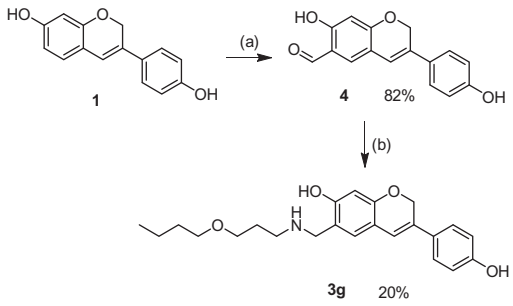


Scheme 6. Reagents and conditions: (a) paraformaldehyde (3 equiv), magnesium dichloride (2 equiv), NEt_3 (2 equiv), 4 h, reflux; (b) 3-butoxypropylamine, $Na(CH_3COO)_3BH$ (1.5 equiv), 3-butoxypropylamine (1 equiv), THF, 48 h, rt, under N_2.

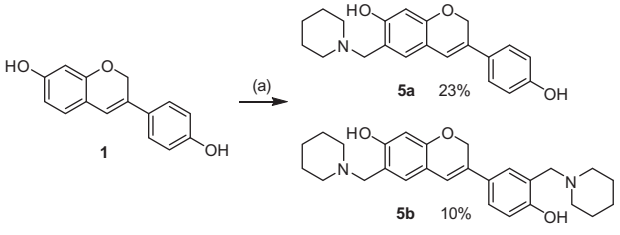
Abdel-Magid et al.[19] reported the use of sodium triacetoxyborohydride as a reducing agent for the reductive amination of aldehydes. Following this literature procedure, the 6-formyl-phenoxodiol was reacted with 3-butoxypropylamine in the same molar ratio of 1:1 in THF at room temperature, under N_2. Sodium triacetoxyborohydride (1.5 equiv) was added to the reaction mixture and stirring continued at room temperature under a N_2 atmosphere for 48 h. The reaction mixture was quenched by adding aqueous 3 N NaOH, which changed the colourless solution to dark green. The mixture was extracted with ethyl acetate, dried and evaporated to give the desired product **3g**.

Mannich reactions of phenoxodiol **1** were also carried out with the secondary amines, piperidine, diethylamine and morpholine. The initial reaction of phenoxodiol **1** with a secondary amine and formaldehyde in molar ratio of 1:2:12 in dioxane, gave multiple products. Subsequently, the quantity of the amine and formaldehyde were reduced to 1.5 and 5 equiv, respectively, but this too resulted in the formation of multiple products. For example, piperidine and formaldehyde were mixed in dioxane at reflux for 30 min. Phenoxodiol **1** was then added to the solution, which was maintained at reflux overnight. Extensive column chromatography was required and led to the isolation of two major products: **5a** (23%) and **5b** (10%) (Scheme 7).

The structure of compound **5b** was further confirmed by X-ray crystallography to be a di-substituted aminomethyl piperidine phenoxodiol (Fig. 1).[20]

The reaction of phenoxodiol **1** with diethylamine yielded similar products **5c** (16 %) and **5d** (5 %). Once again, extensive column chromatography was required to isolate these products (Scheme 8). However, for the morphine reaction, extensive chromatography of the reaction mixture led to the isolation of three products, compound **5e** (30%), compound **5f** (8%) and compound **5g** (50%).

It has been shown in the literature that aminals can function as preformed amine substrates in the Mannich reaction. These highly reactive bis-(dialkylamino)methane derivatives can behave as strong electrophiles and do not require formaldehyde in the aminoalkylation process.[16,17] The aminoalkylation reaction proceeds quickly and produces fewer unwanted side products.[8] The reaction of phenoxodiol **1** with bis(dimethylamine)methane under reflux conditions generated the aminomethyl-substituted Mannich base product **6** in 90% yield (Scheme 9).



Scheme 7. Reagents and conditions: (a) piperidine (1.5 equiv), formaldehyde (5 equiv), dioxane, overnight, reflux.

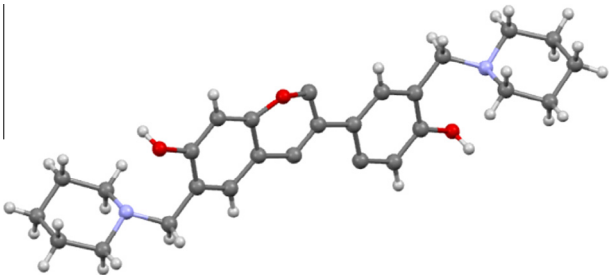
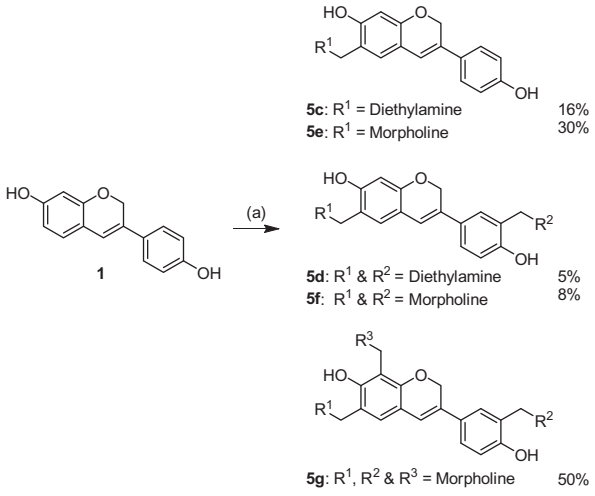


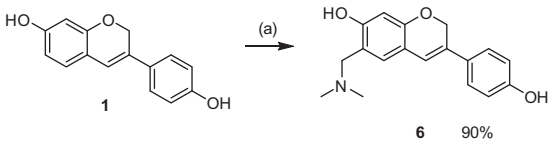
Figure 1. ORTEP diagram of 3-(4-hydroxy-3-(piperidin-1-ylmethyl)phenyl)-6-(piperidin-1-ylmethyl)-2*H*-chromen-7-ol **5b**.



Scheme 8. Reagents and conditions: (a) amine (1.5 equiv), formaldehyde (5 equiv), dioxane, overnight, reflux.

The main objective of the synthesis of Mannich bases was to enhance the potency and specificity against cancer cells of parental compound phenoxodiol **1**. To assess the anti-proliferative properties, the synthesized Mannich bases were grouped into 3 classes (Fig. 2), and their effects on cancer cell proliferation were assessed in vitro using MDA-MB-231 triple-negative breast cancer and SHEP neuroblastoma cell lines.

As shown in Figure 3, all 25 compounds inhibited cancer cell proliferation in a dose-dependent manner. When compared with the parental compound phenoxodiol **1**, both Class 1 and Class 2 displayed improved anti-proliferative activities against MDA-MB-231 breast cancer cells except compound **2b**, which showed similar potency as phenoxodiol **1**. For Class 3, only compounds **5a**, **5b** and **5d** displayed improved anti-proliferative activity against MDA-MB-231 breast cancer cells. Against SHEP neuroblastoma cells, the majority of Class 1 and 2 also showed improved



Scheme 9. Reagents and conditions: (a) bis(dimethylamine)methane, formaldehyde (5 equiv), EtOH, 3 h, reflux.

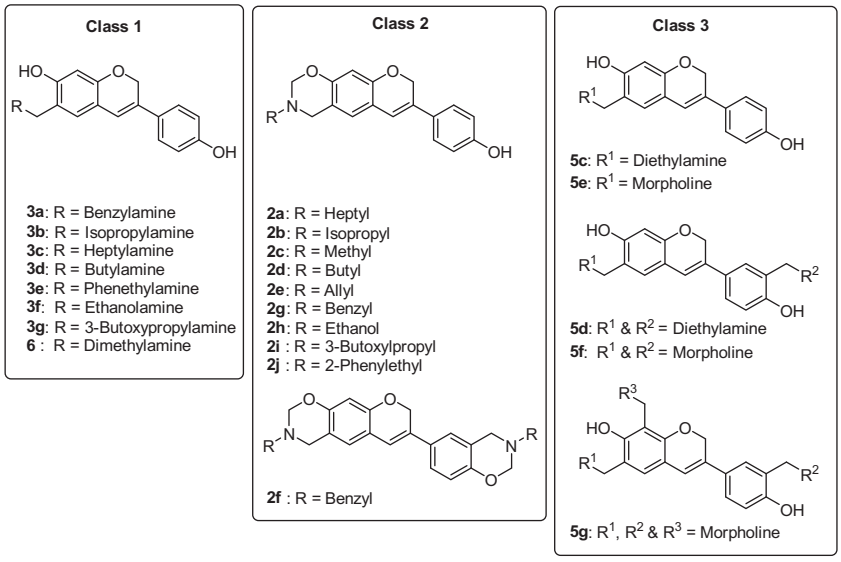


Figure 2. Class 1 Aminomethyl substituted isoflavenes, Class 2 benzoxazine substituted isoflavenes, Class 3 isoflavenes with secondary amine.

anti-proliferative activity with the exception of compounds **2b**, **2e**, **3b**, **2h**, and **3f**. For Class 3, only compounds **5b** and **5d** displayed slightly improved anti-proliferative activity compared to phenoxodiol **1** (Table 1).

Chemotherapy agents target rapidly dividing cells and compounds that are toxic to normal and non-dividing cells are said to be non-specific. To assess the specificity of the Mannich bases, several compounds were selected and tested for their toxicity against normal quiescent cells in vitro using MRC-5 normal lung fibroblasts (Fig. 4).

The specificity of the Mannich bases was calculated using the IC_{50} value for normal cells (MRC-5) divided by the IC_{50} value for the other cell types, according to Eq. 1.

The specificity of phenoxodiol **1** appeared to be cell dependent, with a good specificity against SHEP neuroblastoma cells but a relatively poor specificity against MDA-MB-231 breast cancer cells (Table 2).

Determination of cell line selectivity values,

$$\text{Specificity} = \frac{IC_{50} \text{ of compound against normal cell}}{IC_{50} \text{ of compound against cell line of interest}} \quad (1)$$

Selected compounds **2a**, **d**, **j**, **3a**, **c**, **d**, **e** and **5d** were tested against MRC-5 fibroblasts. All eight compounds showed improved specificity towards MDA-MB-231 breast cancer cells but varying specificity against SHEP neuroblastoma cells compared to parental compound phenoxodiol **1**. Phenylethylamine analogue **2j** followed by **3e** showed highest specificity value against the two cell lines (Table 2).

Phenoxodiol **1** is an isoflavene with potent anti-cancer and chemoresistance modulation properties.[21,22] Our results have shown that the Mannich bases derived from phenoxodiol **1** displayed up to 30 times more potent anti-proliferative activity against the cancer cell lines MDA-MB-231 and SHEP. The synthesized molecules are analogues of phenoxodiol **1** with varying amine substitutions. Hence, a structure–activity relationship (SAR) can be established by comparison of the biological activities of the different phenoxodiol Mannich bases (Table 3).

In the case of the aminomethyl-substituted isoflavenes (Class 1), compounds **3a**, **3c**, **3d**, **3e** and **3g** exhibited improved anti-proliferative activity against both MDA-MB 231 and SHEP cancer cell lines compared to phenoxodiol **1** (Fig. 3). Among these compounds, compound **3c** with an *n*-heptyl side chain and compound **3e** with a 2-phenylethyl side chain exhibited the most potent anti-proliferative activity. In contrast, short chain alkyl amine derivatives such as compounds **6** and **3f** showed the weakest anti-proliferative activity against MDA-MB-231, and compounds **3b** and **3f** showed weak anti-proliferative activity against SHEP. These results demonstrate that the formation of Mannich bases with long chain and bulkier aryl amine groups significantly improved the anti-proliferative activity, whereas, short alkyl side chains or a terminal alcohol did not enhance the anti-proliferative properties of the parent isoflavene **1**.

For benzoxazines (Class 2), compounds **2a**, **2d**, **2j**, **2f**, **2i** and **2g** exhibited superior anti-proliferative activity against cancer cells compared to phenoxodiol **1**. Compounds **2a** and **2j**, which were formed from the same amines as compounds **3c** and **3e**, respectively, exhibited similarly potent anti-proliferative activity suggesting no significant difference in activity between benzoxazines and phenolic aminomethyl analogues. However, compound **2a** a benzoxazine of *n*-heptyl amine was more specific for MDA-MB-231 than compound **3c**, an aminomethyl derivative of *n*-heptyl amine. The difference in specificity between Class 1 and Class 2 compounds is interesting and needs further investigation. Benzylamine analogues, such as compound **2f**, the di-substituted benzoxazine and compound **2g** a mono-benzoxazine had similar anti-proliferative activity, which suggests that both phenolic groups can be substituted. Compounds **2b** and **2h** showed weak anti-proliferative properties, similar to their analogues in Class 1 (compounds **3b** and **3f**, respectively). Additionally, compounds **2c** and **2e** showed only slight improvement in their anti-proliferative activity compared to phenoxodiol **1**. Once again, this demonstrates that short alkyl side chains do not significantly improve the activity. Thus, Class 2 showed a similar trend to that of Class 1 and compounds with long chain and bulkier aryl amine derivatives were more potent anti-proliferative agents.

For isoflavenes with secondary amines (Class 3), only compounds **5d**, a diethylamine analogue, and **5b,** a piperidine analogue, showed more potent anti-proliferative activity than phenoxodiol **1**. Both compounds are di-substituted at C6 and C3′ and are more potent than their mono-substituted counterparts. Interestingly, compound **5f**, a morpholine analogue, showed almost no anti-proliferative effect. This may be due to the poor solubility of this compound. Also, compounds **5e** and **5g** had poor

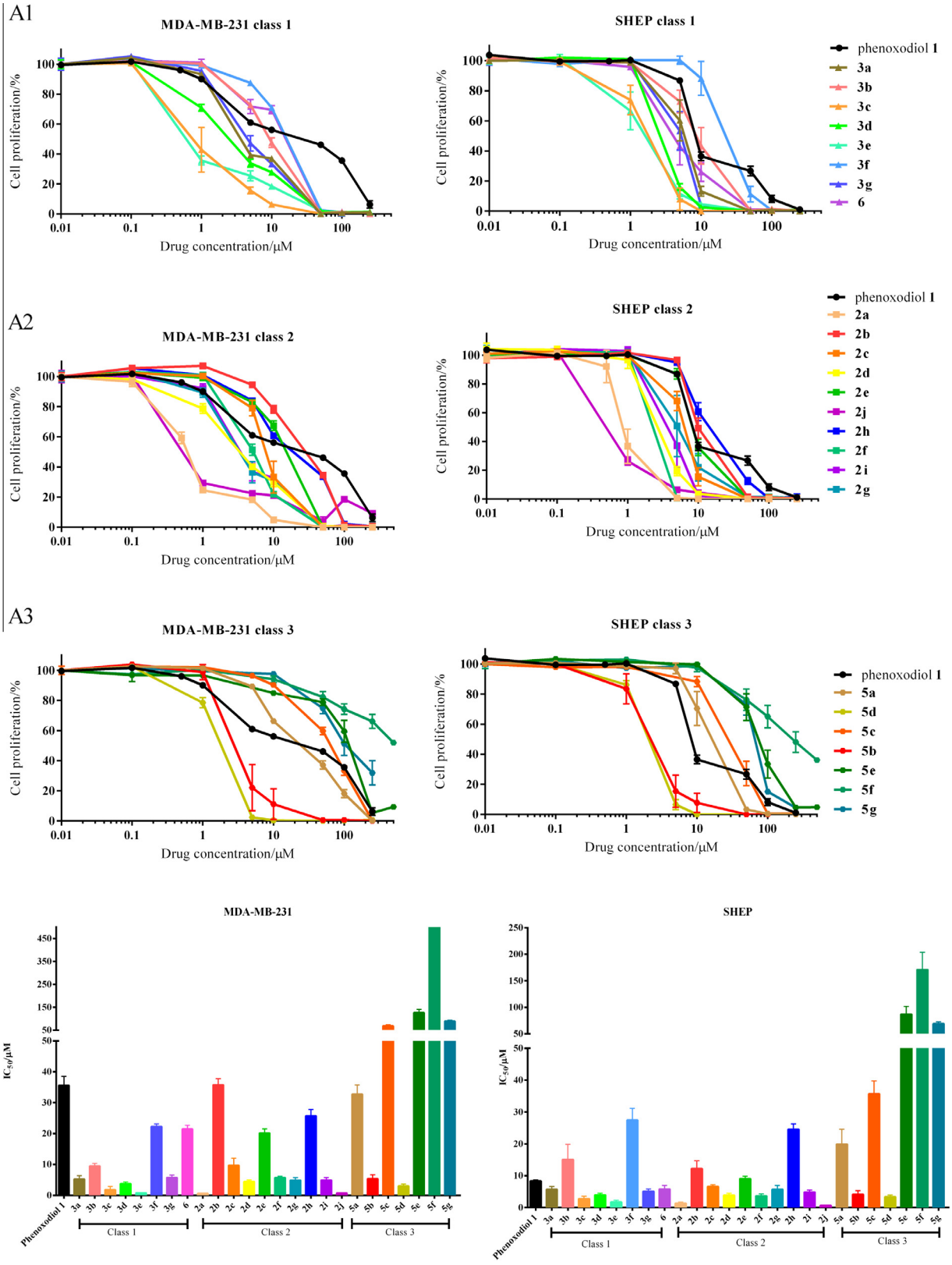


Figure 3. In vitro anti-proliferative properties of phenoxodiol **1** and Mannich bases. MDA-MB-231 or SHEP cells were incubated with a range of concentrations of drug compound for 72 h. (A1–3) Cell proliferation as a function of compound concentration. Points show % of cell proliferation as compared to untreated control cells. Error bars show SE of at least four independent experiments. (B) Histogram representation of IC_{50} values of drug compounds. Error bars show the SE of at least four independent experiments.

activity, which suggests that morpholine has a detrimental impact on anti-proliferative activity.

Steric effects and lipophilicity are two important factors that influence the potency of drug molecules. Lipophilicity affects the

Table 1
IC_{50} values of compounds for MDA-MB-231 and SHEP cells

Class	Compound	IC_{50} (μM)	
		MDA-MB-231	SHEP
Nil	Phenoxodiol 1	35.6	8.4
1	**6**	21.4	5.8
	3a	5.3	5.7
	3b	9.5	15.1
	3c	1.8	2.8
	3d	3.8	4.0
	3e	0.8	1.8
	3f	22.2	27.5
	3g	5.8	5.1
2	**2a**	0.6	1.4
	2b	35.8	12.2
	2c	9.7	6.6
	2d	4.5	3.9
	2e	20.1	9.1
	2f	5.7	3.7
	2g	4.9	5.7
	2h	25.7	24.6
	2i	4.9	4.9
	2j	0.7	0.7
3	**5a**	32.8	19.9
	5b	5.4	4.2
	5c	68.3	35.7
	5d	3.0	3.4
	5e	126.2	86.8
	5f	500.0	171.0
	5g	89.1	69.0

Table 2
IC_{50} values of selected compounds for MRC-5 cells and specificity values for MDA-MB-231 and SHEP cells versus MRC-5 cells

Compound	IC_{50} (μM)	Specificity value	
	MRC-5	MDA-MB-231	SHEP
Phenoxodiol **1**	109	3.0	13.0
2a	11.9	19.8	8.7
2d	33.8	7.6	8.7
2j	197.0	266	274
3a	40.9	7.8	7.1
3c	16.0	9.0	5.8
3d	33.8	8.9	8.5
3e	36.7	45.3	20.9
5d	21.2	7.0	6.3

Table 3
Log*P* value of Mannich bases (calculated from ChemBioDrawUltra 12.0.3)

Class 1	Log*P*	Class 2	Log*P*	Class 3	Log *P*
6	2.65	**2a**	5.44	**5a**	3.38
3a	4.15	**2b**	3.60	**5b**	4.06
3b	2.93	**2c**	2.94	**5c**	3.32
3c	4.76	**2d**	4.19	**5d**	3.94
3d	3.51	**2e**	3.64	**5e**	2.25
3e	4.28	**2f**	6.65	**5f**	1.79
3f	1.75	**2g**	4.68	**5g**	1.33
3g	4.10	**2h**	2.43		
		2i	4.14		
		2j	4.96		

ability of the drugs to cross the plasma membrane while steric hindrance affects the ability of molecules to bind to certain receptors and induce biological responses. Additionally, the synthesized compounds have functional groups such as amine and hydroxyl groups that are prone to metabolism. With increased steric hindrance, the metabolism of the Mannich bases by cellular enzymes may be reduced, thus potentially enhancing the lifetime of the molecules within cancer cells.

Investigations of all analogues were pursued to determine the effect of branched and longer alkyl chains on the potency and specificity of compounds. However, excessive lipophilicity may become an issue as the alkyl chain is increased due to the increase in cellular uptake and retention, potentiating non-specific toxicity. Thus, it would be interesting to understand the effect of various alkyl substituents on lipophilicity, log*P* and anti-proliferative activity.

In order to investigate potential correlations, lipophilicity was plotted against the IC_{50} values of the compounds against MDA-MB-231 and SHEP (Fig. 5).

As shown in Figure 5, there is a significant correlation observed between the lipophilicity of the compounds and their anti-proliferative activity against MDA-MB-231 ($R^2 = 0.23$; p value = 0.015) and SHEP cancer cells ($R^2 = 0.37$; p value = 0.0013). As log*P* increases, the IC_{50} value decreases and therefore the anti-proliferative activity increases. This suggests that lipophilicity plays an important role in determining the activity and selectivity of these Mannich bases against cancer cells.

In summary, a range of isoflavene Mannich bases was synthesized by the reaction of phenoxodiol **1** with various primary and secondary amines. Interestingly, it was observed that varying the concentration of amine, the temperature or the time of reaction led to the formation of different products. Biological assays showed

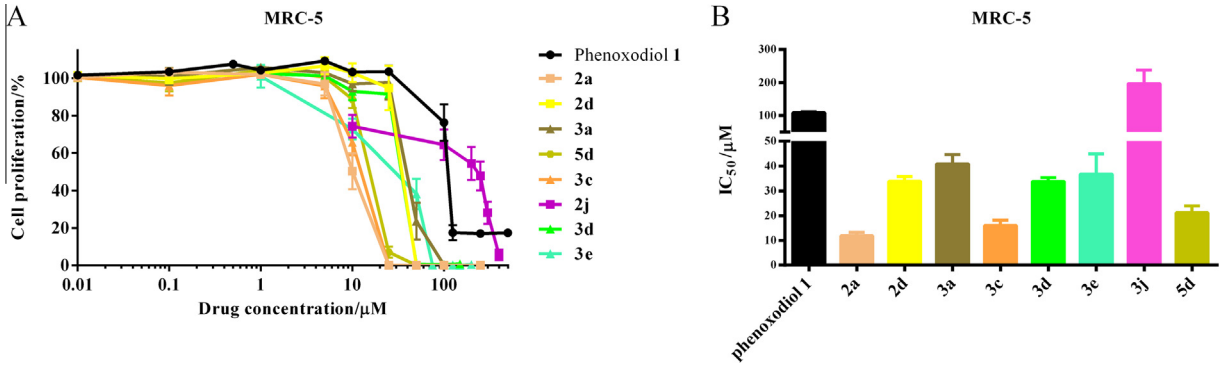


Figure 4. In vitro anti-proliferative properties of phenoxodiol **1*** and compounds **2a**, **d**, **3a**, **c**, **d**, **e**, **j**, and **5d** against MRC-5. (A) Cell proliferation as a function of compound concentration. Points show % of cell proliferation as compared to untreated control cells. Error bars show SE of at least four independent experiments. *At concentrations above 100 μM the media becomes saturated with phenoxodiol **1**, resulting in a plateauing of the activity. (B) Histogram representation of IC_{50} values of drug compounds. Error bars show the SE of at least four independent experiments.

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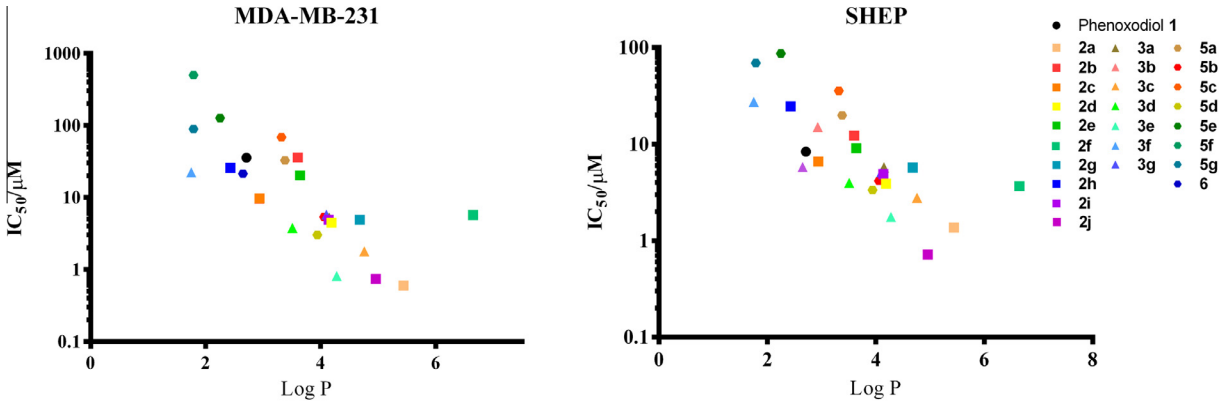

Figure 5. Plot of log*P* versus IC_{50} (μM) for MDA-MB-231 and SHEP.

that the isoflavene Mannich bases exhibited higher antiproliferative activities against MDA-MB-231 breast cancer and SHEP neuroblastoma cancer cells compared to the parent compound phenoxodiol **1**. Notably, the most potent molecule **2j** showed strong potency against both cancer cell lines and low toxicity against MRC-5 normal human lung fibroblasts, with specificity values of 266 and 274 for MDA-MB-231 and SHEP cell lines, respectively. Further analysis is therefore needed to determine the mode of action of compound **2j**.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.09.027.

References and notes

1. Kennecke, H.; Yerushalmi, R.; Woods, R.; Cheang, M. C.; Voduc, D.; Speers, C. H.; Nielsen, T. O.; Gelmon, K. *J. Clin. Oncol.* **2010**, *28*, 3271.
2. Bray, F.; Ren, J. S.; Masuyer, E.; Ferlay, J. *Int. J. Cancer* **2013**, *132*, 1133.
3. Ferlay, J.; Soerjomataram, I.; Dikshit, R.; Eser, S.; Mathers, C.; Rebelo, M.; Parkin, D. M.; Forman, D.; Bray, F. *Int. J. Cancer* **2015**, *136*, E359.
4. Torre, L. A.; Bray, F.; Siegel, R. L.; Ferlay, J.; Lortet-Tieulent, J.; Jemal, A. *CA Cancer J. Clin.* **2015**, *65*, 87.
5. Mense, S. M.; Hei, T. K.; Ganju, R. K.; Bhat, H. K. *Environ. Health Perspect.* **2008**, *116*, 426.
6. Gruber, C. J.; Tschugguel, W.; Schneeberger, C.; Huber, J. C. *N. Engl. J. Med.* **2002**, *346*, 340.
7. Gamble, J. R.; Xia, P.; Hahn, C. N.; Drew, J. J.; Drogemuller, C. J.; Brown, D.; Vadas, M. A. *Int. J. Cancer* **2006**, *118*, 2412.
8. Bondarenko, S. P.; Levenets, A. V.; Frasinyuk, M. S.; Khilya, V. P. *Chem. Nat. Compd.* **2003**, *39*, 271.
9. Wang, D.; Hou, L.; Wu, L.; Yu, X. *Chem. Pharm. Bull. (Tokyo)* **2012**, *60*, 513.
10. Heaton, A.; Kumar, N. Eur. Patent WO 2000AU00103 20000215, **2000**.
11. Heaton, A.; Kumar, N.; Kelly, G. E.; Husband, A. Eur. Patent WO2000AU01056 20000906, **2001**.
12. Heaton, A.; Kumar, N.; Kelly, G. E.; Husband, A. U.S. Patent 20090233999, **2009**.
13. Heaton, A.; Kumar, N.; Walker, C. Eur. Patent WO2008AU00267 20080229, **2008**.
14. Husband, A. J.; James, M.; Kumar, N. Eur. Patent WO2009AU00973 20090730, **2010**.
15. Kukhareva, T. S.; Krasnova, V. A.; Koroteev, M. P.; Kaziev, G. Z.; Kuleshova, L. N.; Korlyukov, A. A.; Antipin, M. Y.; Nifant'ev, E. E. *Russ. J. Org. Chem.* **2004**, *40*, 1190.
16. Garazd, Y. L.; Panteleimonova, T. N.; Garazd, M. M.; Khilya, V. P. *Chem. Nat. Compd.* **2002**, *38*, 532.
17. Garazd, Y. L.; Panteleimonova, T. N.; Garazd, M. M.; Khilya, V. P. *Chem. Nat. Compd.* **2003**, *39*, 330.
18. Hofslokken, N.; Skattebol, L. *Acta Chem. Scand.* **1999**, *53*, 258.
19. Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849.
20. *Crystal data*: $C_{27}H_{31}N_2O_3$, M.W. = 435.26, *P*21/*c*, cell dimensions: *a* = 12.518(3), *b* = 6.3300(13), *c* = 14.363(3). Crystallographic data excluding structure factors have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 1407654. A copy of the data can be obtained free of charge from CCDC, 12 Union Road, Cambridge CB2 IEZ, UK or email: deposit@ccdc.cam.ac.uk.
21. Howes, J. B.; de Souza, P. L.; West, L.; Huang, L. J.; Howes, L. G. *BMC Clin. Pharmacol.* **2011**, *11*, 1.
22. Kamsteeg, M.; Rutherford, T.; Sapi, E.; Hanczaruk, B.; Shahabi, S.; Flick, M.; Brown, D.; Mor, G. *Oncogene* **2003**, *22*, 2611.