A phenotypic screen identifies a compound series that induces differentiation of acute myeloid leukemia cells *in vitro* and shows anti-tumour effects *in vivo*

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Abstract

Induction of differentiation is a promising therapeutic strategy against acute myeloid leukemia. However, current differentiation therapies are effective only to specific patient populations. To identify novel differentiation agents with wider efficacy, we developed a phenotypic high-throughput screen with a range of genetically diverse cell lines. From the resulting hits, one chemical scaffold was optimised in terms of activity and physicochemical properties to yield OXS007417, which was also able to decrease tumour volume in a murine *in vivo* xenograft model.

Introduction

Acute myeloid leukemia (AML) is the most aggressive type of blood cancer, and the second most common leukemia in adults. AML arises from genetic mutation(s) which cause an arrest of differentiation at the progenitor or precursor stage, blocking production of downstream blood lineages. This leads to the accumulation of immature myeloid cells – blasts – in the bone marrow and peripheral blood, which disrupts normal haematopoiesis.¹ AML can occur at all ages, but predominantly affects elderly people, with 80% of patients being over 60 years of age.^{2,3}

AML can originate from accumulation of different combinations of genetic abnormalities, which underlies the highly heterogeneous nature of the disease. Different AML subtypes have been described, with the FAB⁴ and the WHO⁵ classifications being the most common ones. The standard of care for many years has been intensive chemotherapy with cytarabine in

combination with anthracycline-derived antibiotics such as daunorubicin to induce apoptosis of AML blasts, which often cannot be tolerated by elderly patients. Other cytotoxic targeted therapies have had little success, as most select for specific clones, leading to selection pressure and resistance or relapse.

An alternative to cytotoxic agents is differentiation therapy. While initial attempts failed to induce clinical remission,^{6,7} a breakthrough in clinical oncology was achieved when all-trans-retinoic acid (ATRA) was shown to release myeloid blasts from differentiation arrest in the subset of AML patients with acute promyelocytic leukemia (APL). Previously APL had been the worst prognostic subsets of AML with complete remission (CR) rates of 40% after chemotherapy,⁸ however treatment with a combination of ATRA and arsenic trioxide induced CR in 90% of patients.⁹ Although this represents only 10% of the AML patient population, these encouraging results have resulted in growing interest in differentiation therapy research in AML.¹⁰ In the recent years, new therapeutic agents capable of overcoming the differentiation blockade have been described, such as DHODH,^{11,12} IDH1 and IDH2,^{13,14} FLT3¹⁵ and LSD1^{16,17} inhibitors, some of which target specific mutations. These examples suggest that inducing differentiation in AML could be more effective and less toxic than current therapies, however novel agents are needed which are effective for wider or more refractive patient populations.

To identify new agents capable of inducing differentiation to AML blasts, which do not target a specific mutation or patient subpopulation, a phenotypic medium throughput assay was developed and used to screen a library of drug-like small molecules. From the resulting hits, a medicinal chemistry optimisation program was initiated, ultimately to provide OXS007417. This small molecule was capable of inducing differentiation to a range of AML cells *in vitro*, had favourable pharmacokinetic properties and reduced tumour volume in a murine AML xenograft *in vivo* model.

Materials and methods

Experimental procedures can be found in the Supplementary Information file.

Results

A phenotypic assay identifies novel small molecules that can differentiate AML cell lines

To find new small molecules that can differentiate AML cells, the cell-surface marker CD11b was chosen as it is upregulated upon myeloid differentiation regardless of the lineage that the cells specialise into. Three AML cell lines representing different population subtypes (HL-60, THP-1 and OCI-AML3) were treated with a compound library of 1,000 small molecules. Cells were stained with CD11b-PE and DAPI, and upregulation of CD11b in the live cells was detected via flow cytometry. For the initial screen, compounds were dosed at 10 μ M for 4 days. The compounds that upregulated CD11b in all three cell lines by more than 10% were selected for further investigation. Firstly, to detect autofluorescent compounds, which could show an increase in fluorescence independent of CD11b upregulation, cells treated with the compounds were stained with isotype-PE. This would also identify compounds showing a non-specific

increase of the antibody. Differentiation ability was then further investigated through morphology analysis and assessment of cell number and viability following compound treatment. These were performed in six AML cell lines with high genetic diversity (HL-60, THP-1, OCI-AML3, Kasumi-1, KG-1 and ME-1).

Those compounds causing a specific CD11b increase, changes to morphology indicative of differentiation, and decrease of cell numbers in all six cell lines were resynthesised. The compounds where the biological activity was confirmed with the authentic solid sample were considered to be confirmed hits.

Figure 1. Screen workflow from primary assay to confirmed hits.

One of the confirmed hits of the screen was imidazopyridine OXS000675 (Figure 2A), which induced differentiation in all six AML cell lines (Figure 2B-F, Figures S1-S8). After a four-day treatment, OXS000675 increased CD11b by up to 92%, with EC₅₀ values of 2.3 – 7.6 μ M. The morphology of the cells treated with OXS000675 showed clear changes indicative of differentiation as compared to negative controls, such as a pronounced increase in size and granularity. Also, the number of viable cells stopped increasing after 24 h, consistent with a block in proliferation.

A

B

F

OXS000675 was weakly active in the CD11b assay (4.8 μ M in HL-60 cells), had good aqueous solubility (>200 μ M) and had reasonable metabolic stability in mouse S9 (mS9) liver fraction ($t_{1/2}$ = 42 min). Its overall profile was deemed to be a good starting point for a medicinal chemistry programme. The initial optimisation was focussed on biological activity of the analogues evaluated in HL-60 cells only, and an initial assessment of physicochemical properties was based on metabolic stability and aqueous solubility. The metabolic stability of the analogues was assessed in mouse liver S9 fraction, and is indicated as extraction ratio (ER).¹⁸

OXS000675 and analogues were easily accessed in 4 steps from 2,4-dichloro-5-nitropyridine (Scheme 1).¹⁹ The imidazopyridine N-1 substituent was first introduced by regioselective nucleophilic aromatic substitution of an amine on the 4-chloro position of the nitropyridine. Subsequent reduction of the nitro group and cyclisation with triethylorthoformate provided imidazopyridine **4**. This could then be derivatised at the C-6 position via either Buchwald-Hartwig or Suzuki-Miyaura coupling to give **5** and **6** respectively.

Scheme 1. Synthetic route to analogues of OXS000675

Initial SAR studies on the N-1 position of the imidazopyridine core established that removal of the methylene linker was highly beneficial for activity, providing OXS007014 with a 35-fold increase in potency, but at the expense of metabolic stability. Pleasingly, a favourable level of solubility was retained (Table 1).

Table 1. Structure-Activity Relationships of N-1 analogues

Compound	R_2	EC_{50} [nM]	Solubility [μM]	ER (mS9)

OXS000675		4800	>200	0.2
OXS007014		140	>200	1

At the C-6 position, another significant gain in potency was achieved when the amine linker was removed, and the aryl was substituted in the *ortho* position. Both *o*-methyl and methoxy groups were well tolerated. An additional fluorine atom in the *para* position led to another significant enhancement in activity, taking compounds OXS007287 and OXS007184 into the low nM range (EC_{50} = 15 and 35 nM). This is in striking contrast to OXS007317 and OXS07268, which have the fluorine in the *meta* position instead and are only weakly active.

Table 2. Structure-Activity Relationships of C-6 analogues

Compound	R ₁	EC ₅₀ ^a [nM]	Solubility ^b [μM]	ER ^c (mS9)
OXS007014		140	>200	1
OXS007746		6900	>200	0.58
OXS007266		>30000	>200	0.19
OXS007034		>30000	146	0.21
OXS007136		1600	28	1
OXS007142		69	>200	1

OXS006988		140	>200	1
OXS007316		2500	157	1
OXS007287		15	>200	1
OXS007184		35	42	1
OXS007317		2500	157	1
OXS007268		1300	9	1
OXS007823		780	n.d. ^d	n.d.
OXS007267		3000	n.d.	n.d.

^a Half maximal effective concentration of %CD11b response in HL60 cells; ^b Semi-thermodynamic aqueous solubility; ^c Extraction ratio (ER)= intrinsic clearance / species flow rate (mice: 90 ml/min/kg) in mouse S9 fraction (mS9); ^d Not determined.

Despite the promising activity of these compounds, all the examples had high clearance in mS9 (ER 1). The most potent group on C-6 (*o*-Me-*p*-F, OXS007287) was chosen for the next generation of analogues, where attention was focussed on improving metabolic stability whilst maintaining activity (Table 3). It was hypothesised that this could be achieved through modification of electronic properties on the aromatic ring attached to N-1, aiming to reduce the overall electron density and substitute one or both of the putatively metabolically labile methoxy groups.

The *m*-methoxy group was found to not be required for biological activity; its removal led to a slight increase in potency, but unfortunately not in metabolic stability (OXS007372). Substitution of the *m*-position with either electron withdrawing (OXS007373, OXS008397) or sterically bulky (OXS007787) groups was not beneficial either. Adding nitrogens to the N-1 aromatic ring (OXS007749, OXS007837, OXS007785) improved metabolic stability, however this was at the cost of reduced biological activity. Encouragingly though, it was found that replacing the *p*-methoxy with a *p*-OCHF₂ (OXS007417) gave a large improvement in metabolic stability, whilst causing only a modest drop in activity (EC₅₀ = 48 nM). It was found that further reducing the electron density at this key group by incorporating an extra fluorine

atom (OCF₃, OXS007951) greatly reduced the activity. It was also found that replacement of the OCHF₂ group with a structurally dissimilar electron withdrawing group such as a sulfone (OXS007839) eliminated biological activity, despite having an improved stability and solubility profile. OXS007417 seemed to give the best balance between biological activity and metabolic stability. The OCHF₂ group appears to be a useful middle ground reducing the analogue's susceptibility to metabolic oxidation, whilst preserving some accessibility of the oxygen lone pairs for bonding interactions.

The 1-methylindazole group was found to be an excellent bioisostere of the methoxy group, providing the highly potent OXS007570 with EC₅₀ = 2 nM. However, it displayed poorer solubility and higher metabolic clearance than OXS007417.

Table 3. Structure-Activity Relationships of N-1 analogues

Compound	R ₂	EC ₅₀ ^a [nM]	Solubility ^b [μM]	ER ^c (mS9)
OXS007287		15	>200	1
OXS007372		6	117	1
OXS007370		19	12	0.97
OXS007392		36	75	1
OXS007540		3	122	1

OXS008411		140		
OXS007839		>30000	>200	0.07
OXS007417		48	36	0.18
OXS007951		2500	n.d. ^d	n.d.
OXS007373		10	49	1
OXS008397		21	n.d.	n.d.
OXS008444		14	n.d.	n.d.
OXS007787		>10000	n.d.	n.d.
OXS007749		70	>200	0.39

OXS007801		>10000	n.d.	n.d.
OXS007571		370	161	0.09
OXS008495		30	n.d.	0.66
OXS007837		3700	16	0.66
OXS007785		2900	>200	0.11
OXS007812		>30000	n.d.	n.d.
OXS007570		2	15	0.57

^a Half maximal effective concentration of %CD11b response in HL60 cells; ^b Semi-thermodynamic aqueous solubility; ^c Extraction ratio (ER)= intrinsic clearance / species flow rate (mice: 90 ml/min/kg) in mouse S9 fraction (mS9); ^d Not determined.

With the more metabolically stable analogues in hand, substituent tolerability on the C-6 position was further analysed (Table 4).

At the *ortho*-position, the methyl group gave the best activity. Analogues containing a chloro OXS008296, methoxy OXS007739 and propoxy OXS008318 groups were also tolerated, while electron withdrawing groups (CF₃, OCF₃) led to a significant decrease in potency. At the *para* position, chloro substituted OXS007930 was equivalent to fluoro in terms of activity, but the latter was preferred as it was less lipophilic. Cyano containing OXS007556 was also well tolerated, and provided a further increase in metabolic stability and solubility. Pyridine

derivatives OXS007865 and OXS007864 were prepared in an effort to reduce clogP, but the additional nitrogens were not tolerated.

Table 4. Structure-Activity Relationships of second-generation C-6 analogues

Compound	R ₁	EC ₅₀ ^a [nM]	Solubility ^b [μM]	ER ^c (mS9)
OXS007417		48	36	0.18
OXS008278		10000	n.d. ^d	n.d.
OXS007739		93	3	0.21
OXS007924		980	n.d.	n.d.
OXS008296		90	n.d.	n.d.
OXS008316		1500	n.d.	n.d.
OXS008297		6900	n.d.	n.d.
OXS007928		2900	n.d.	n.d.
OXS008318		58	n.d.	n.d.
OXS008317		1100	n.d.	n.d.

OXS007929		180	n.d.	n.d.
OXS007556		370	68	0.02
OXS007930		28	n.d.	n.d.
OXS008161		1000	n.d.	n.d.
OXS008187		890	n.d.	n.d.
OXS007733		220	15	0.15
OXS007865		2000	n.d.	n.d.
OXS007864		1200	n.d.	n.d.

^a Half maximal effective concentration of %CD11b response in HL60 cells; ^b Semi-thermodynamic aqueous solubility; ^c Extraction ratio (ER)= intrinsic clearance / species flow rate (mice: 90 ml/min/kg) in mouse S9 fraction (mS9); ^d Not determined.

The importance of the *ortho* group on the C-6 aryl unit is remarkable. It is clearly evidenced by comparing the non-*ortho* substituted OXS007136 and OXS008278, with the corresponding *o*-methyl-substituted OXS007142 and OXS007417, which are 23- and 21-fold more active respectively. It was hypothesised that this jump in potency could be due to steric interference between the methyl group and the imidazopyridine core, inducing a molecular twist which would reduce planarity in the molecules. This conformational change caused an advantage not only in activity, but also a gain in solubility, consistent with a less productive crystal packing of the less planar molecules. To provide further evidence, DFT optimisations were performed on OXS007417 and OXS008278. As shown on Figure 3, the methyl group of OXS007417 is predicted to cause rotation of the aryl group, increasing the dihedral angle between the core and the LHS, thus conveying greater three dimensional character onto the molecule. In an attempt to increase the dihedral angle, molecules with 2,6-substitution (OXS007924) or bulkier *ortho* groups (OXS008317) were prepared, but these led to a decrease in activity, suggesting that there was a size limitation within the target binding site.

Figure 3. Predicted conformations of OXS007417 and OXS008278 from DFT energy minimisation. The dihedral angles between the bicyclic core and the N-1 and C-6 aryl groups are shown.

Substitution of the C-2 position of the imidazole was not well tolerated (Table 5).

Table 5. Structure-Activity Relationships of C-2 analogues

Compound	R ₃	EC ₅₀ ^a [nM]	Solubility ^b [μM]	ER ^c (mS9)
OXS007752		10000	71	0.3
OXS007768		3800	6	0.1

^a Half maximal effective concentration of %CD11b response in HL60 cells; ^b Semi-thermodynamic aqueous solubility; ^c Extraction ratio (ER)= intrinsic clearance / species flow rate (mice: 90 ml/min/kg) in mouse S9 fraction (mS9).

Overall, OXS007417 gives the best balance of high potency, with reasonable metabolic stability and solubility, and was therefore chosen as a lead molecule for progression into further *in vitro* and *in vivo* studies (Figure 4). In spite of the moderate solubility at neutral pH, there was an improvement in acidic buffer (pH 1), probably due to the basic nature of the imidazopyridine core.

A more detailed *in vitro* ADME assessment of OXS007417 was undertaken. It showed reasonable stability in mouse hepatocytes (ER = 0.31), high cellular permeability measured in a Caco-2 assay (P_{app} = 14 x10⁻⁶ cm/s) and high plasma protein binding (PPB, 99% bound).

Figure 4. Overview of evolution from the hit OXS000675 to the *in vivo* lead molecule OXS007417.

OXS007417 differentiates AML cell lines *in vitro*

Following the optimisation of the original hit molecule OXS000675 into OXS007417, it was important to verify that the lead molecule continued to be effective across multiple AML cell lines, and displayed a mechanistic profile consistent with differentiation.

Gratifyingly, CD11b flow cytometry analysis of THP-1 and OCI-AML3 cells treated with OXS007417 showed similar values to HL-60 (Figure 5A,B). Also, OXS007417 caused a clear decrease in cell numbers in the panel of six AML cell lines (Figure 5C). In some of the cell lines, and in particular ME-1, the decrease in viability was small, consistent with induction of differentiation and block in proliferation, rather than direct apoptosis. OXS007417 also caused morphological changes consistent with differentiation, such as a lighter cytoplasm, higher cytoplasm to nucleus ratio, increase in granulation and change on cell shape and size (Figure 5E-H and Figures S9-S14).

A

B

Cell line	EC ₅₀ [nM]
HL-60	48
OCI-AML3	87
THP-1	118

C

D

Figure 5. *In vitro* activity of OXS007417. A) Concentration-response curves of %CD11b determined by flow cytometry, HL-60, OCI-AML3 and THP-1 cells treated with OXS0007417, and B); C) Number of live cells per well and D) %viability of HL-60 cells treated with either DMSO control or 370 nM OXS007417 over 4 days, determined with acridine orange and PI; Morphology of HL-60 cells treated with E) DMSO control, F) OXS0007417 370 nM; Morphology of THP-1 cells treated with E) DMSO control, F) OXS0007417 370 nM

OXS007417 has favourable pharmacokinetic properties and is well tolerated at a therapeutic dose

Next, the pharmacokinetic profile of OXS007417 was evaluated in male CD-1 mice. In the first study, OXS007417 was dosed at 3 mg/kg PO, and 1 mg/kg IV (Figure 6A, Table 5). The compound had moderate clearance (64 ml/min/kg) and volume of distribution (4.6 L/kg), leading to an elimination half-life of 1.5 h, and reasonable bioavailability (48%). At 3 mg/kg, the C_{max} was 128 ng/mL, which is 7-fold higher than the EC_{50} *in vitro*, and compound concentration above the EC_{50} was maintained for 5 h. It should be noted though that the high protein binding determined for OXS007417 (99%) means that the free drug available is likely to be significantly less than this.

Figure 6. Pharmacokinetic characterisation of OXS007417. A) Observed blood-concentration profile in mice following single 3 mg/kg PO and 1 mg/kg IV dosing of OXS007417; B) Observed blood-concentration profile in mice following 60 mg/kg PO dosing of OXS007417 at days 1 and 4; C) Simulated exposure of OXS007417 at 30 mg/kg BID.

Table 5. Pharmacokinetic parameters of OXS007417 determined in the single dose 3 mg/kg, and repeat dose 60 mg/kg studies

Parameter	3 mg/kg single dose	60 mg/kg repeat dose	
		Day 1	Day 4
$T_{1/2}$ ^a (h)	1.5		8.1
AUC_{0-8h} ^b (ng/mL.h)	363	9860	9225
$AUC_{0-8h}/dose$ (ng/mL.h /(mg/kg))	121	164	154
C_{max} ^c (ng/ml)	128	2212	2675
$C_{max}/dose$ (ng/mL/(mg/kg))	43	37	45
Cl_{obs} ^d / F ^e (ml/min/kg)	129		
F (%)	48		

^a Elimination half-life; ^b Area under the curve; ^c Maximum concentration; ^d Clearance; ^e Bioavailability.

To maintain constant exposure levels above EC₅₀ (48 nM = 18 ng/mL), repeat dosing at a higher compound dose was contemplated. A repeat dose study at 60 mg/kg was undertaken in CD-1 mice, where compound levels were measured on days 1 and 4 (Figure 6B). The study confirmed linear PK between the 3 and 60 mg/kg dose levels (Table 5), as indicated by both AUC and C_{max}. The half-life, however, was significantly increased at 60 mg/kg, which could be indicative of saturation of clearance, or could reflect slower absorption of drug at the higher dose level. The similar exposure levels at days 1 and 4 indicated no accumulation of compound over time.

To estimate an appropriate dose level, the observed blood concentration-time data were fit to a single compartment PK model (Figure S15, see supplementary information for details) and then used to simulate the exposure over 1 week at 30 mg/kg twice daily (Figure 6C). The predicted values showed that at 30 mg/kg BID the compound exposure levels would be constantly maintained above 10xEC₅₀. For subsequent efficacy studies, a dose of 10 mg/kg twice daily was considered to be appropriate to sustain sufficient exposure levels, whilst mitigating effects of protein binding.

In subsequent tolerability studies, dosing of compound for 7 days at 10 mg/kg BID was well tolerated with no apparent adverse effects.

Overall, PK and tolerance studies identified 10 mg/kg BID as a suitable efficacy testing dose for OXS007417, and demonstrated viability of maintaining exposure levels above EC₅₀.

OXS007417 reduces tumour volume in an HL-60 subcutaneous xenograft model

To study the ability of OXS007417 to inhibit tumour growth *in vivo*, a subcutaneous xenograft model was used. HL-60 cells were implanted onto the flank of female NOD SCID mice, and tumours were allowed to reach a volume of 150 mm³ before start of treatment. OXS007417 was administered PO at 3 mg/kg BID, 10 mg/kg QD and BID, and 30 mg/kg BID for 4 weeks. Cyclophosphamide (IP, 150 mg/kg Q5D), a clinical cytotoxic drug, was used as an internal control.

Treatment with OXS007417 was well tolerated at 3 and 10 mg/kg and did not lead to significant body weight loss. At 10 mg/kg twice daily dosing, OXS007417 significantly delayed the growth of HL-60 tumours, with T/C (ratio of tumour volume in treated mice versus control) of 57%, when compared to vehicle group (p<0.0001). A clear dose-dependent response was observed: at 10 mg/kg once daily dosing, the T/C was 79%, thus with a significant slowing of the growth of the tumours when compared to vehicle (p=0.0155), but to a lesser extent than at twice daily dosing. Tumours in 3 mg/kg BID treated animals followed a similar pattern to those treated with vehicle (p=0.3637).

Treatment at 30 mg/kg had a striking effect on tumour growth, almost diminishing tumour volume entirely on day 7. However, this dose was not well tolerated, and this arm of the study was discontinued at Day 9.

At the end of the study, blood samples were taken for bioanalysis at 1, 4 and 8 h following the final dose treatment. Exposure of OXS007417 was detected in all treatment groups; it was dose-dependent and decreased over time following compound administration. The exposure levels were also in agreement with those measured in the earlier PK studies.

Figure 7. Evolution of tumour volume in the HL-60 subcutaneous model (female NOD SCID mice). Values shown are mean $\pm$ SD; n=10 at day 0 for all vehicle and OXS007417 groups, and n=5 for the cyclophosphamide group.

Discussion

Despite recent advances in the treatment of AML, resistance and relapse following treatment is still a major problem, and novel therapeutic approaches are imperative. Differentiation therapy is a promising alternative to the traditional cytotoxic chemotherapy; however, clinical application has so far only been successful in a small subset of patients. The high heterogeneity of the patients with AML challenges the search for a “one fits all” drug, but a differentiation agent effective in all AML patients regardless of their mutation status would be a major breakthrough.

In this work, a medium throughput phenotypic screen was developed to identify novel small molecules capable of differentiating AML cells. Phenotypic assays are particularly attractive in the search for drugs without a predefined target. To ensure that the effect was not limited to a specific patient population, a range of cell lines representing a variety of AML subtypes were utilised. CD11b was chosen as a marker of differentiation, and its upregulation was detected by flow cytometry. The hits were confirmed by assessing their effect on cell proliferation and morphology. One of the confirmed hits was OXS000675, which was effective in differentiating the panel of 6 cell lines, albeit with relatively modest potency (EC_{50} 2.2 – 7.8 μ M).

A medicinal chemistry programme was undertaken to improve the potency and physicochemical properties of OXS000675. Removal of the amine and methylene linkers at C-6 and N-1 respectively led to a significant increase in potency. At the C-6 position, *ortho*-substitution was also critical for activity, and a *para*-fluorine atom was also beneficial. The

importance of the *ortho* substituent was attributed to a twist of the molecule, which was supported by DFT calculations.

Despite the promising potency of these initial compounds, they lacked sufficient metabolic stability for further progression. Detailed investigation of the N-1 aromatic ring revealed that the OCHF₂ group was capable of reducing electron density, thus increasing stability, while maintaining high activity.

The most promising compound OXS007417 was confirmed to induce differentiation *in vitro* in 6 AML cell lines, and it was progressed to *in vivo* studies. Pharmacokinetic studies at 3 mg/kg and 60 mg/kg, as well as tolerability studies, identified that dosing at 10 mg/kg twice daily would be a suitable dose.

In a subcutaneous study with HL-60 cells, treatment with OXS007417 at 10 mg/kg BID resulted in significant delay of tumour growth. A lower dose (3 mg/kg BID) was not effective, and a higher dose (30 mg/kg QD) was not well tolerated. Overall, this study identified an effective dose where OXS007417 was capable of inducing significant antitumour activity without adverse effects.

Conclusion

In summary, a phenotypic screen was developed to identify novel small molecules capable of differentiating AML cells. A medicinal chemistry programme to optimise one of the hit molecules identified OXS007417, which induced differentiation of a range of AML cell lines *in vitro*. OXS007417 had favourable pharmacokinetic properties and showed anti-tumour effects *in vivo* in an AML subcutaneous model.

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Conflict of Interest Statement

PV, TM, SGD and AJR are founders and minor shareholders of OxStem Oncology.

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