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Title

Design, Synthesis and Evaluation of the Anti-Cancer Activity of 2-Amino-aryl-7-aryl-benzoxazole Compounds.

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Short Title: Anti-Cancer Activity benzoxazole inhibitors

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Abstract

A series of 2-aminoaryl-7-arylbenzoxazole derivatives have been designed, synthesized and evaluated as anti-cancer agents. Fourteen of the compounds exhibited cytotoxic effects towards Human A549 lung cancer cells. We found **12l** was the most potent with an EC_{50} of 0.4 μM, equivalent to the anti-cancer drug doxorubicin, but had low selectivity following cross screening in monkey kidney Vero cells. Eight of the most potent or most selective compounds were further profiled in additional cell lines (MCF7, NCI-H187 and KB) to better understand their cytotoxic activity. Only compound **12l** had a measurable EC_{50} in a single cell line (3.3 μM in the KB cell line). Taken together, this data suggests the series as a whole display specific cytotoxicity towards A549 cells. Cheminformatics searches pointed to JAK2 as a possible target. A subset of compounds assayed at this target showed IC_{50}s ranging from 10 μM to 0.08 μM, however no clear correlation between JAK potency and A549 cytotoxicity was observed.

Introduction

According to the World Health Organization, lung cancer is the most common cause of cancer death.[1] Non-small cell lung cancer (NSCLC) is the most deadly form of this disease and has a 5 year survival rate below 15% [2]. Platinum-containing chemotherapies,[3] DNA intercalators such as Doxorubicin and Etoposide[4] and protein kinase inhibitors such as Lapatinib and Imatinib [5] can be used to treat NSCLC. This range of treatment options is necessary to reduce patient specific side-effects [6] and to avoid disease resistance in certain sub-populations [7]. Nevertheless, the low survivability associated with lung cancer is a clear indication of the need for more effective drugs, particularly those that act on targets or pathways of greater relevance to the disease. Kinases inhibitors have been of immense interest to the pharmaceutical industry[5] due to the critical role these targets play in cellular signaling processes, including cell growth, differentiation and death. [8] Specific signaling and regulatory pathways are often overexpressed in cancers leading to tumor growth and blocking these pathways is a recognized strategy to target the disease [9]. Kinases are now the most intensively studied class of molecular targets in drug discovery [10] with approximately 30 new inhibitors on the market.[11] In NSCLC, the tyrosine kinases EGFR and VEGFR [7] are established targets used to tackle this specific disease. Other kinases are currently under

investigation including JAK inhibitors [12, 13]. The JAK/STAT pathway has been identified as a potential intervention point [3, 12, 14-16] and has seen JAK inhibitors entering clinical trials.[12, 16]

In this study, we report the anti-cancer activity of 2-aminoaryl-7-arylbenzoxazole compounds at an NSCLC cell line (Figure 1). Compounds **1**-**3** were obtained from the focused screening of approximately 100 diverse kinase-like compounds in an A549 based cytotoxicity assay. This manually curated set consisted of low molecular weight compounds with the typical donor-acceptor interaction features required for kinase ATP site binding. The 2-aminoaryl-7-arylbenzoxazole series was selected on the grounds of its good activity and low MWT (Figure S1), synthetic tractability, and based on previous reports of the scaffolds kinase activity. Substructure and similarity searching identified 2-amino-7-aryl-benzoxazole derivatives and related 2-Amino-[1,2,4] triazolo [1,5-a]pyridines as JAK2 inhibitors which have been used to target leukemia (Figure 2). [17, 18] Our observation of activity in an NSCLC cell line is consistent with reports that the JAK/STAT pathway represents an intervention point in lung cancer [3, 12, 14-16]. RSK2 is also a possible target [19], however the IC_{50}s associated with this target are at the sub micromolar level compared to the nano- to sub-nanomolar level of the former (Figure S2).

We undertook alterations at the 2- and 7- positions guided by the SAR of the initial hits (Figure 1) and knowledge from previous literature reports. We selected aryl substituents for the 7-position (R^1) of benzoxazole scaffold that were primarily hydrophobic in nature (Figure 1). In an effort to reduce the overall hydrophobicity of the molecules, relatively polar substituted aniline reagents were selected for substitution at the 2-position of benzoxazole scaffold (R^2). While kinase biochemical data is available in the literature for this chemotype, very little phenotypic data has been reported. The primary goal of this study has been to shed light on the cytotoxic properties of this scaffold, to improve our SAR understanding the series with a view to obtaining more potent molecules. We have assessed the cytotoxicity of the series further using the monkey kidney epithelial cell line (Vero), the MCF7 breast cancer cell line, the SCLC NCI-H187 cell line and the KB cell line. In addition, an assessment of the activity of a subset of compounds at JAK2 kinase have also been undertaken.

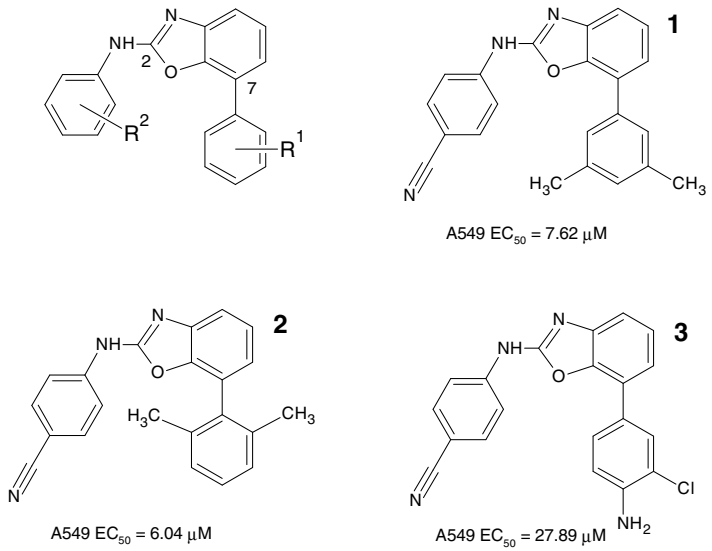


Figure 1: Structurally related screening hits identified from cytotoxicity screening in the A549 cell line.

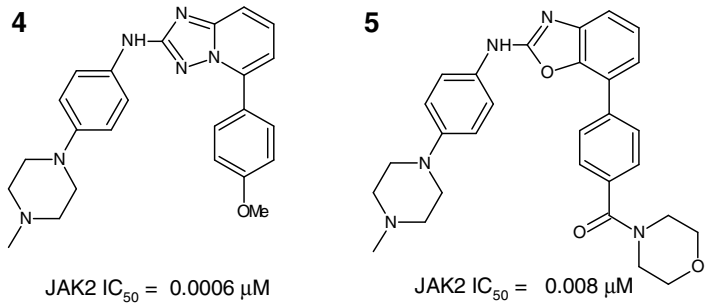


Figure 2: Nanomolar active amino-7-aryl-benzoxazole kinase inhibitors reported in the literature [17, 19].

1 Experimental Section

1.1 Compound Design

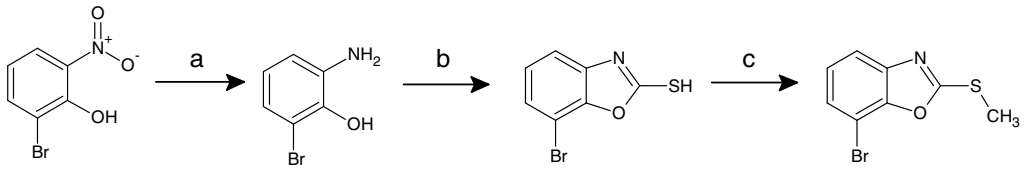
From our initial screening, compounds **1** and **2** display low μM activity towards A549, however they also have unacceptably high clogPs of 6. **3** has lower lipophilicity, but lower A549 activity also. From the outset, incorporation of polarity at the R^2 position was therefore considered a higher priority in the overall effort to improve physiochemical properties. As 2-amino-7-aryl-benzoxazole derivatives and related 2-Amino-[1,2,4] triazolo [1,5-a]pyridines have are reported as JAK2 inhibitors. As such consideration was given to how molecules would bind to the ATP binding site of the protein kinase JAK2 given this is a probable target. Compounds were docked to JAK2 via (a) manual superposition

onto the 1,2,4-triazolo[1,5-a]pyridin-2-amine of JAK2 (PDB 4JIA).[18, 20] Computational docking using GOLD5.1 was also undertaken. All solvent and co-factors were removed and compounds docked with default settings.[21]

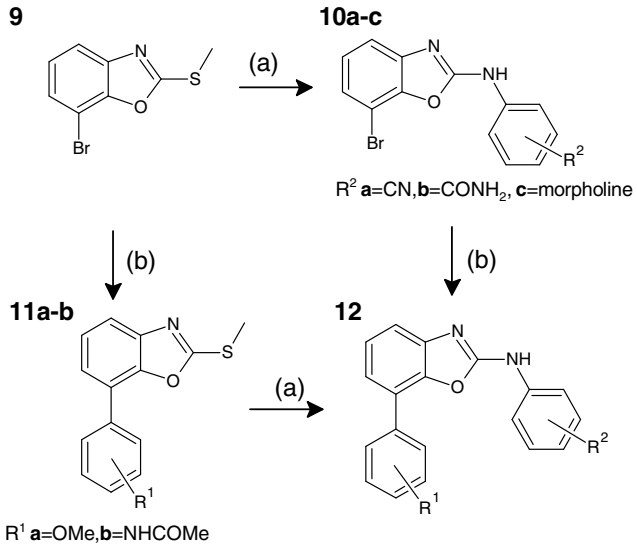
The expansion of the SAR around our hits was achieved by designing structural modifications to (a) lower the overall lipophilicity of the series, (b) explore the hydrophobic/hydrophilic balance between the R^1 and R^2 positions guided by property considerations and structural understanding of JAK2 binding mode (c) focusing on substituents that allow us to explore novel chemical space of the series. Reagent selection was primarily driven by a desire to reduce the hydrophobicity at either the R^1 and R^2 positions (Scheme 2), or both, in order to improve the overall molecular properties.

1.2 Chemical Synthesis

All chemicals received from suppliers were used without further purification. All reactions were monitored by thin layer chromatography aluminum/silica gel plate with visualization of UV light. Column chromatography was performed by irregular silica gel (60Å). Mass spectra (MS) were obtained on an Agilent 1100 HPLC instrument coupled to a LC/MSD Trap mass spectrometer, in ESI (+) mode or APCI mode and on a Bruker micrOTOF-Q III in ESI (+) mode. Compounds showed a purity ≥ 95% as determined from the corresponding UV visible absorbance HPLC chromatogram. ^{1}H and ^{13}C NMR spectra were recorded on a Varian instrument at 400 MHz and 101 MHz. Chemical shifts are reported in ppm (δ) using the residual solvent line as the internal standard. Compound synthesis followed the general routes described in Scheme 1 and Scheme 2 with full details being provided in the supporting information.



Scheme 1: General route to 7-bromo-2-methylsulfanyl-benzoxazole (**9**): (a) $SnCl_2$, EtOH, (b) Potassium ethylxanthogenate, EtOH, (c) K_2CO_3, MeI, DMF [22].



Scheme 2: Synthetic approach used for the preparation of final compounds from **9**.

1.2.1 Preparation of 7-bromo-2-methylsulfanyl-benzoxazole (9)

A mixture of 2-bromo-6-nitrophenol (**6**) (18.4 mmol) and tin (II) chloride dihydrate (91.7 mmol) in EtOH (80 ml) was heated at 70 ^{o}C for 1 h. The mixture was poured into ice and the pH was made slightly basic (pH 7-8) by addition of 1N sodium hydroxide solution. The aqueous solution was extracted with EtOAc. The extract was washed with brine, dried over magnesium sulfate and concentrated under vacuum to afford of the title compound 2-Amino-6-bromophenol (**7**), 40% yield.

2-amino-6-bromo-phenol (**7**) (26.6 mmol) was dissolved in 20 ml EtOH and (39.9 mmol) potassium ethylxanthogenate was added. This mixture was stirred at reflux for 6 h. After it had cooled down to room temperature, the reaction mixtures were concentrated in vacuum and 50 ml water was added along with the addition of acetic acid, to obtain a pH of 5. The precipitate was filtered off, washed with water and dried to afford the title compound **8**, 90% yield.

A mixture of (26.1 mmol) 7-bromo-benzoxazole-2-thiol (**8**), (52.2 mmol) K_2CO_3, (28.7 mmol) MeI in 80 ml, DMF was stirred at room temperature for 1 h. The reaction mixture was poured into water and extracted with EtOAc. The combined organic layers were washed with water and saturated NaCl solution, dried over $MgSO_4$, and the filtrate was concentrated in vacuum to afford the title compound **9**[22], 30% yield.

1.2.2 General procedure for amine substitution (Step a)

To 7-bromo-2-methylsulfanyl-benzoxazole (**9**) in DCM, m-CPBA was added. This mixture was stirred at room temperature for 1 h. It was then diluted in sat. $NaHCO_3$, water and brine. After that it was dried by anhydrous Na_2SO_4, filtered and concentrated. Then aniline was added and the reaction mixture was heated to 40°C with stirring for 20 h. The reaction mixture was concentrated in vacuum and the crude was purified by irregular silica gel (60Å) column chromatography using a mixture of EtOAc and hexane to give **10a-c**.

1.2.3. General procedure for boronic acid coupling (Step b)

A solution of **9** and phenyl boronic acid were dissolved in dioxane. A solution of Na_2CO_3 (in 5 ml of water) was added and a stream of N_2 (g) was bubbled through the mixture in order to exclude oxygen from the reaction mixture. Tetrakis (triphenylphosphine) palladium was added and the reaction mixture was stirred at 100 ^{o}C for 24 h. After that, the reaction mixture was poured into water and was extracted with EtOAc. The combined organic layers were washed with water and dried over $MgSO_4$. It was purified by an irregular silica gel-G60 column chromatography using a system of EtOAc/hexane or MeOH/DCM to give **11a-b**.

1.2.4 General Procedure for the preparation of compound 1-3, 12a-12m

The preparation of compounds **1-3, 12h, 12l** and **12m** used intermediates **10a-c** which were reacted with the phenyl boronic acid using the general procedure described in section 1.2.3. The preparation of compound **12a-12g** and **12i-12k** used intermediates **11a-b** reacted with anilines using the general procedure described in 1.2.2 (Scheme 2).

1.3 Biological Testing

1.3.1 Cytotoxicity at A549 and Vero cell lines

The EC_{50} of compounds were accessed in A549 (ATCC CCL-185) and Vero (ATCC CCL-81) cell lines using the MTT method by Mosman *et.al.*,[3, 23, 24] Cells were incubated in DMEM medium with 10% fetal bovine serum, 100 U/ml of penicillin and streptomycin at 37

^{o}C in a humidified atmosphere with 5% CO_2. The cells were incubated in DMEM for 16-18 h, after with test compound for 72 h in triplicate. Then, to each well was added DMEM containing 3-[4, 5-dimehyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide (MTT), and cells were incubated for a further 3 h. Subsequently, 50 µl dimethyl sulfoxide was added. The plate was shaken, and then absorbance readings at a wavelength of 570 nm were performed on a Sunrise microplate reader (Tecan). Doxorubicin was used as the positive control and 0.5% DMSO as the negative control. The EC_{50} values of compounds were derived from dose-response-curve plotted between %cytotoxicity versus the sample concentrations fitted using Prism 6.0 GraphPad Software Inc., (San Diego, CA, USA).

1.3.2 Cytotoxicity at MCF7 NCI-H187 KB cell lines

The cytotoxicity against the MCF7 (ATCC HTB-22), NCI-H187 (ATCC CRL-5804) and KB cell lines (ATCC CCL-17) using the method described by Brien *et.al,.*[25] Cells were grown and maintained in a complete medium and incubated at 37 ^{o}C humidified incubator with 5% CO_2. Briefly, to a 384-well plate in triplicate, 5 µl of test compound and 45 µl of cell suspension were added. The plate was then incubated at 37 ^{o}C in a humidified incubator with 5% CO_2 for 72 h. After that, resazurin solution was added to each well and the plate was further incubated at 37 ^{o}C for 4 h. Fluorescence was monitored at 530 nm (excitation) and 590 nm (emission) using bottom-reading mode. Ellipticine and Doxorubicin were used as the positive control and 0.5% DMSO as the negative control. The EC_{50} value was derived from dose-response-curve that is plotted between %cytotoxicity versus the sample concentrations by using SOFTMax Pro software (Molecular Devices, USA).

1.3.3 JAK2 kinase activity

Kinase assays were performed as previously described.[24] Reactions were performed using 10 ng JAK2 enzyme, 0.1 mg/ml poly (Glu:Tyr) substrate and 0.1 mg/ml ATP. Inhibition was determined by using Antibody Beacon^{TM} Tyrosine Kinase Assay Kit (A-35725; Invitrogen, USA) 4-aryl-N-phenylpyrimidin-2-amine [26] was used as the positive control and 0.5% DMSO as the negative control. The IC_{50} value was derived from dose-response-curve plotted between % cytotoxicity versus the sample concentrations by using GraphPad Software Inc. (San Diego, CA, USA).

2 Results

2.1 Synthesis

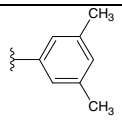
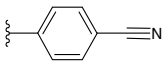
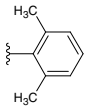
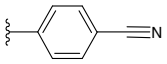
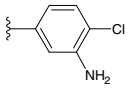
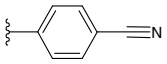
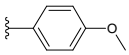
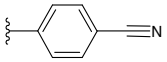
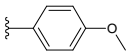
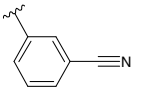
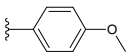
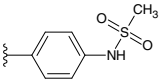
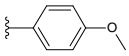
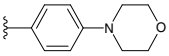
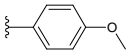
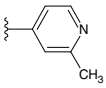
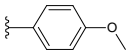
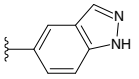
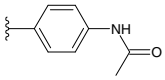
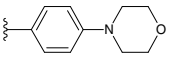
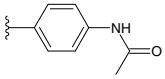
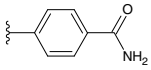
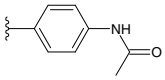
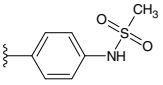
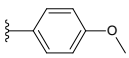
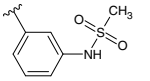
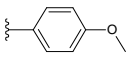
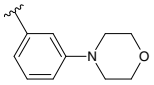
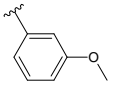
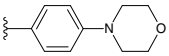
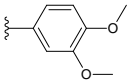
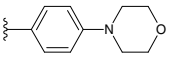
2-amino-aryl-7arylbenzoxazole derivatives have been synthesized using the general synthetic procedure described in Scheme 1 and Scheme 2. The hydrogenation reaction of 2-bromo-6-nitrophenol (**6**) was carried out using tin (II) chloride dehydrate to give 2-amino-6-bromo-phenol (**7**) (40% yield). This was followed by ring closure in the presence of potassium ethyl xanthogenate to give 7-bromo-benzoxazole-2-thiol (90% yield) (**8**). Intermediate compound bromo-2-(methylsulfanyl)-1, 3-benzoxazole (**9**) was obtained from the reaction of compound **8** and MeI (38% yield).

1-3, 12h, 12l and **12m** were produced in two steps, starting with the coupling of 7-bromo-2-methylsulfanyl-benzoxazole (**9**) with anilines by activating the sulphur leaving group using m-CPBA to give **10a-c**. Suzuki-Miyaura cross-coupling of **10a-c** with phenylboronic acid utilized Pd $(PPh_3)_4$ and Na_2CO_3 under a nitrogen environment. We investigated changing the order of the substitution and coupling reactions to overcome the lack of reactivity for certain reagent combinations. **12a-g** and **12i-k** were produced in two steps starting with the coupling of boronic acids with **9**, leading to the formation of **11a-b**, followed by aniline substitution at the 2-position. Overall, the yields ranged from 3-30% which is in line with reports elsewhere. [19]

2.1 Biological Testing

The anti-cancer properties (EC_{50}) of all synthesized compounds in A549 and Vero cell lines are reported in Table 1. Also reported are computed physico-chemical properties and the selectivity index (SI), the latter representing the selectivity compounds show for A549 over Vero cells. The cytotoxicities of the most potent or more selective exemplars were evaluated in the MCF7, NCI-H187 and KB cell lines and these results are reported in Table 2. Finally, the IC_{50}s associated with a sub-set of the compounds were evaluated in a JAK2 inhibition assay and are reported in Table 2.

Table 1: Activities and compounds 1-3, 12a-m

ID	R^1	R^2	clogP[a]	MWT[a]	A549 EC_{50} (μM)	Vero EC_{50} (μM)	SI
1			6.0	339	7.6 ±3.4	11.9±0.5	1.6
2			6.0	339	6.0±1.7	8.6±2.7	1.4
3			4.8	361	27.9±4.1	41.4±23.9	1.5
12a			4.9	342	12.4±0.2	0.6±0.06	0
12b			4.8	342	3.6±0.8	2.3±0.04	0.6
12c			3.5	409	1.2±0.5	>100	80.0
12d			4.9	401	1.8±0.3	3.0±0.6	1.7
12e			3.9	332	>100	>100	-
12f			4.4	356	1.6±0.02	30.4±15.9	19.0
12g			4.5	428	16.4±1.9	8.9±0.3	0.5
12h			3.3	386	>100	>100	-
12i			3.1	436	31.8±25.7	>100	3
12j			3.5	410	13.0±0.8	6.4±0.2	0.5
12k			4.8	401	6.7±0.6	0.8±0.01	0.1
12l			4.9	401	0.4±0.01	0.8±0.02	2.0
12m			4.7	431	3.2±0.9	2.5±0.9	0.8
Doxorubicin			1.4	544	0.4±0.08	1.4 ±0.1	-

[a] Calculated logP and MWT using JChem Version 14.9.100.707.[27]

Table 2: Activities of compounds towards additional cell lines and JAK2 kinase.

ID	% inhibition at 10 μM / [EC_{50}, μM] MCF7	NCI-H187	KB	JAK2 IC_{50} μM
12b	37.1 ±1.7 / [-]	0.0±0.1 / [-]	0.0±0.1 / [-]	n.d.[a]
12c	41.3±4.7 / [-]	12.9±0.4 / [-]	8.3±0.1 / [-]	4.9±0.15
12d	0.0 ±0.4 / [-]	0.0±0.9 / [-]	0.0±0.1 / [-]	0.08±0.04
12f	13.7 ±0.2 / [-]	0.0±0.1 / [-]	0.0±0.1 / [-]	n.d.[a]
12g	43.1±4.3 / [-]	1.7±0.1 / [-]	7.4±0.1 / [-]	0.11±0.05
12i	0.0±0.1 / [-]	26.9±0.4 / [-]	0.0±0.1 / [-]	10±0.08
12l	43.8 ±3.6 / [-]	25.9 ±2.0 / [-]	90.8 ±11.5 / [3.29]	n.d.[a]
12m	0.0±0.2 / [-]	20.7±0.6 / [-]	0.0±0.1 / [-]	n.d.[a].
Ellipticine	-	99.2± 7.4 / [3.9]	99.7±14.6 / [11.4]	n.d.[a]
Doxorubicin	93.1± 6.6 / [16.5]	96.9±8.7 / [0.11]	96.3± 18.6 / [2.5]	n.d.[a]
4-aryl-N-phenyl pyrimidin-2-amine [28]	n.d.[a]	n.d.[a]	n.d.[a]	0.06±0.01

[a] Activity not determined (n.d.).

4 Discussion

4.1 Cytotoxicity SAR

The A549 anti-cancer values ranged from 0.4 μM to >100 μM. **12l** was the most potent of all compounds with an EC_{50} of 0.4 μM. This was almost equivalent to the known NSCLC anti-cancer drug doxorubicin (EC_{50} = 0.4 μM). Compounds **12c**, **12d**, and **12f** all inhibited the A549 cell line with EC_{50}s below 2 μM (1.2, 1.6, and 1.8 μM respectively). Two additional compounds had EC_{50}s between 6-7 μM, five compounds had EC_{50}s between 7-50 μM (original hits **1**-**3**, **12a**, **12g**, **12i** and **12j**), while the final two compounds had EC_{50}s > 100 μM (**12e & 12h**).

At the R^1, incorporation of phenyl groups with methoxy at the 3- position, 4- position, or both was undertaken due to the previously discussed JAK2 SAR. Compound **1** and **2** showed reasonable activity in A549 cells. The introduction of the 4-OMe phenyl at R^1 (**12a**) results in a desirable drop in clogP (6.02 to 4.8), however the A549 activity also drops by approximately 2 fold. Incorporation of additional polar anilines at the R^2 position was undertaken (**12b-12f**). The 3-CN-phenyl of **12b** was found to be 3.4 fold more potent than the equivalent molecule substituted at the 4-position (**12a**). Substituting the R^2 phenyl for 4- methyl-sulphonamide and 4-morpholine also results in a dramatic improvement in potency, with EC_{50}s values of 1.2 and 1.8 μM being observed. 1H-indazol-6-amine (**12f**) resulted in a comparable EC_{50} of 1.6 μM while the methyl-pyridine of **12e** was inactive. This could be rationalized on the grounds that the more polar derivatives can interact more effectively at or around the active site pocket of the postulated kinase target, as well as potentially from the improved solubility improving the cell based response.

We next investigated the use of acetamide at R^1, this having both donor and acceptor features available for target interaction (**12g-12j**). With 4-Morpholine, 4-amide and 4-methylsulphonamide at the R^2, relatively poor activity was observed showing a subtle balance exists in terms of the hydrophobic/hydrophilic balance needed to achieve activity. Significant polarity at both R groups, while potentially beneficial in terms of overall lipophilicity, and solubility, appears detrimental to overall activity. Compounds **12j** differs from **12c** in that the N-methyl-sulphonamide substitution is at the 3- rather than the 4-position. This change results in a 10 fold loss in potency. Modification of **12d** in a similar manner results in a 3 fold loss in potency suggesting the 4-position substitution is preferred. Two additional compounds were prepared with 3-MeO-phenyl and 2, 3 di-MeO-phenyl at R^1 and 4-substituted morpholine at R^2. The former resulted in compound **12l**, the most potent observed here with an EC_{50} of 0.4 μM, while the latter had a potency of 3.2 μM.

Certain classes of cancer drugs can show broad cytotoxicity across a number of cell lines. [6, 29] We therefore assessed all compounds against the monkey kidney epithelial cell line (Vero) (Table 1). The most potent compound observed was **12a** (0.6 μM). Doxorubicin, a relatively non-specific DNA intercalator, was observed to have an EC_{50} of 1.4 μM. Compounds **12k** and **12l** have Vero cell EC_{50} values of 0.80 μM, while compounds **1, 2, 12b, 12d, 12g, 12j** and **12m** displayed EC_{50}s in range 3.6-11.9 μM. The SAR associated with the Vero cell-line broadly follows that for A549. However, there are three noticeable outliers in compounds **12c**, **12f** and **12i**, which display noticeably greater selectivity towards A549 cells. These compounds were characterized as having generally lower lipophilicity values (mean clogP = 3.6, N=3) than their more non-selective, A549 active counterparts (mean clogP = 4.9, N=11).

Eight compounds were evaluated in the three additional cell lines NCI-H187 (small cell lung cancer), MCF7 (breast cancer), and KB (oral cavity cancer) and it was found that the majority of the compounds showed only minimal cross-activity. Only compound **12l** displayed a measurable EC_{50} at the KB cell line (3.3 µM). Compounds **12c** and **12g** showed a degree of inhibition (~40% inhibition at the highest concentration screened) in the MCF7 cell line. This would suggest a more specific mechanism of activity for these compounds, which is consistent with the hypothesis that these are functioning as kinase inhibitors.

4.2 Role of JAK2 Inhibition

The results in Table 2 show that the series shows some JAK2 activity, in two cases at the sub µM level. Compounds were docked into a JAK2 crystal structure (4JIA) [18] for visual assessment using a scaffold based superposition of the benzoxazole ring onto the triazolopyrimidine scaffold of the inhibitor bound to the protein (Figure 3). The compounds appear to be classic hinge-binders, making two H-bonds with Leu932 (Figure 2). The 7-positon phenyl ring binds in the hydrophobic back pocket and the 2-position aniline group is directed towards the ATP pocket opening (Figure S3).

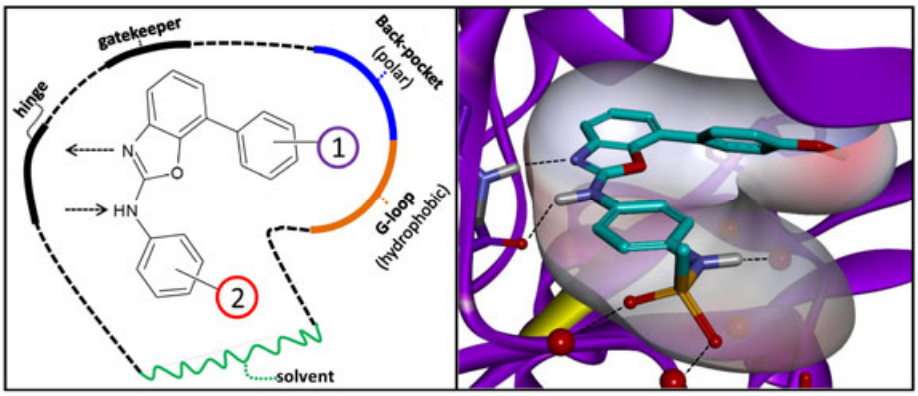


Figure 3: Configuration of JAK2 binding site (left) and the 3D docked model of **12c** bound to the JAK2 X-ray crystal structure (4JIA, Purple) [17]. The hinge region of JAK2 is also shown in stick form and H-bonds are indicated by dotted lines.

The SAR data shows that **12d** showed the most significant inhibition of JAK2 with an IC_{50} 0.08 µM. The 4-phenyl-morpholine is a classic group incorporated by other JAK2 classes at this portion,

including 4-aryl-2-aminoalkylpyrimidines.[28] **12g** also displayed reasonable activity at 0.11 µM while **12c** and **12i** displayed IC_{50}s of 4.9 and 10 µM respectively. It would be inappropriate to conclude that JAK2 activity is the primary cause of the observed cytotoxicity [3, 12, 14-16] of these compounds in A549 cells given the relatively limited correlation with the cell based response. Further detailed studies will be needed to ascertain the target(s) associated with these compounds.

5 Conclusions

We have designed and synthesized sixteen compounds of 2-amino-aryl-7-arylbenoxazole derivatives and assessed their biological activities at a range of cancer cell lines: A549, Vero, MCF7, NCI-H187, KB cell lines. **12l** displayed the best anti-cancer properties at the A549 cell line with an EC_{50} value of 0.4 µM. Compound **12c** displayed the best balance of A549 activity (1.2 µM) and selectivity towards other cell lines (SI = 80). The most potent, or most selective compounds, were further profiled in additional cancer cell lines (MCF7, NCI-H187 and KB). Only compound **12l** displayed a measurable EC_{50} (3.3 µM) in KB cell line confirming the series displays relatively low general cytotoxicity overall, suggesting a more specific mode of action.

Biochemical testing at a possible target, JAK2 kinase, confirmed the chemotypes activity towards this recognized NSCLC target.[3, 12, 14-16] **12d** showed the most significant potency at JAK2 (0.08 µM). The compounds as a whole display good physicochemical properties; MWT mean of 382 with a maximum of 436; clogP mean of 4.5, with only 2 examples with clogP values above 5 (the initial hits **1**, **2**). Further detailed studies are needed to better understand the anti-cancer potential of these compounds.

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Notes

The authors confirm no conflicts of interest.

Figure legends

- **Figure 2:** Structurally related screening hits identified from cytotoxicity screening in the A549 cell line.
- **Figure 2:** Nanomolar active amino-7-aryl-benzoxazole kinase inhibitors reported in the literature [17, 19].
- **Figure 3:** Configuration of JAK2 binding site (left) and the 3D docked model of **12c** bound to the JAK2 X-ray crystal structure (4JIA, Purple) [17]. The hinge region of JAK2 is also shown in

stick form and H-bonds are indicated by dotted lines.

- **Scheme 1:** General route to 7-bromo-2-methylsulfanyl-benzoxazole (9): (a) SnCl2, EtOH, (b) Potassium ethylxanthogenate, EtOH, (c) K_2CO_3, MeI, DMF [21].
- **Scheme 2:** Synthetic approach used for the preparation of final compounds from **9**.

Supplementary Material

Additional experimental details and characterization data of selected compounds have been provided.