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Synthesis and evaluation of the NSCLC anti-cancer activity and physical properties of 4-aryl-*N*-phenylpyrimidin-2-amines

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ABSTRACT

Reported herein are efforts to profile 4-aryl-*N*-phenylpyrimidin-2-amines in terms of their anti-cancer activity towards non small-cell lung carcinoma (NSCLC) cells. We have synthesized new 4-aryl-*N*-phenylpyrimidin-2-amines and assessed them in terms of their cytotoxicity (A549, NCI-H187, MCF7, Vero & KB) and physicochemical properties ($logD_{7.4}$ and solubility). **13f** and **13c** demonstrated potent anti-cancer activity in A549 cells (0.2 μM), compared to 0.4 μM for the NSCLC drug Doxorubicin. **13f** also displayed low experimental $logD_{7.4}$ (2.9) and the best solubility (~40 μM). Compounds **13b** and **13d** showed the best balance of A549 anti-cancer activity and selectivity. **13g** showed good activity and selectivity comparable with the anti-cancer drug Doxorubicin.

Lung cancer is one of the largest contributors to cancer fatalities in the world according to the World Health Organization (WHO).[1] The incidence rates are highest in less developed regions, and generally higher in men compared to women. It is found that the incidence rates in different regions of the world reflect different historical exposures to tobacco smoking.[1,2] There are two main types of lung cancer: Non small-cell lung carcinoma (NSCLC) and small-cell lung carcinoma (SCLC), the former accounting for approximately 80% of the total.[3–5] Recent estimates put its five-year survival rate at ~15% confirming the need for improved therapeutic treatments.[6]

A number of targeted treatments exist for NSCLC. Target based approaches have dominated drug discovery due to benefits associated with selectively modulating a particular target relevant to the disease pathway; ease of automation as well as its high throughput nature.[7] Protein kinases offer great potential due to their role in regulating the majority of cellular pathways, including cellular growth, differentiation and apoptosis.[8] These inhibitors work on certain patient sub-populations and are sensitive to gene mutations,[9,10] meaning new of treatments are constantly sought. Current kinase inhibitors used to treat NSCLC include Erlotinib, Lapatinib (EGFR) and Crizotinib and Ceritinib (ALK).[8]

In this work we investigate the anti-cancer activity of 4-aryl-*N*-phenylpyrimidin-2-amines. These compounds are known to act at a range of targets (Fig. S1), including exemplars in Fig. 1 that target protein kinases.[11–14]

Substructure searches performed on the ChEMBL database using the *N*-phenyl-4-(aryl)pyrimidin-2-amine core reveals 2599 unique target-based activities (532 unique compounds) and 476 unique cell based cytotoxicities (184 unique compounds). Cytotoxicity measurements represent 34% of total measurements but were collected on just 18% of the total compounds. The advantage of taking a cell based approach is the perceived increased disease relevance[15–19] and the possibility of identifying compounds with desirable polypharmacology becomes more likely.[20,21]

Less decorated 4-aryl-*N*-phenylpyrimidin-2-amines, as exemplified by the structures in Fig. 1, act on targets previously reported to have implications for NSCLC. JNK plays a role in mediating cell growth and self renewal in A549 cells.[22–27] It was also reported CDK5 regulates the proliferation, motility and invasiveness of lung cancer in A549 cells[28] and that it was a potential prognostic biomarker for NSCLC.[29] IKKβ depletion in tumor cells significantly attenuated tumor proliferation and significantly prolonged mouse

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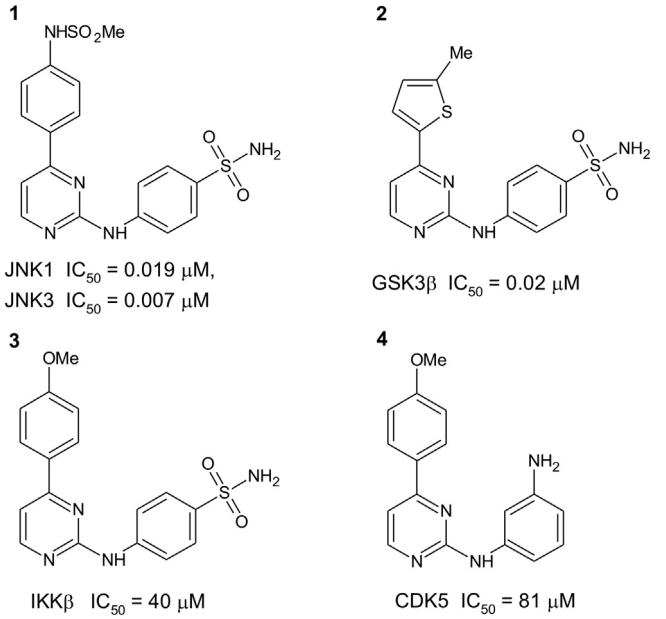

Fig. 1. A selection of 2-phenylamino-pyrimidine compounds targeting JNK2,[11] IKK2,[12] GSK3[13] and CDK5.[14]

survival[30] and its selective targeting was reported to potentiate the efficacy of Taxol in NSCL cancer.[31] It was also reported that maintaining GSK3 cross activity was needed to maintain the effectiveness of mTOR inhibitors.[32] Preparation of new compounds **5** and **6** (Fig. 2) was therefore undertaken and these were subsequently shown to be micro-molar inhibitors of the human adenocarcinomic alveolar basal epithelial cells (A549) prompting a more extensive investigation.

Herein, we report the design, synthesis and biological evaluation of new 4-aryl-*N*-phenylpyrimidin-2-amine compounds. Our goal is to improve our SAR understanding of this series by screening additional analogs in the A549 cell line making subtle modifications to scaffold and evaluating their cytotoxicity, and the overall physical properties.[33–35] We also assess the cytotoxicity of the series using monkey kidney epithelial cell line (Vero).[36] For the most effective compounds, the activity at additional human cell lines is also evaluated; SCLC cell line (H187); HeLa contaminated cervical adenocarcinoma cells cell line (KB); breast cancer cell (MCF7), as are the experimental logD and phosphate buffer solubility at $pH_{7.4}$.

A selection of new compounds were prepared Modification to the 2-position and the 4-position of the pyrimidine scaffold were undertaken as shown in Table 1. Intermediates **7**–**12** were prepared as detailed in Scheme 1 using established synthetic methods.[11,37,38] This intermediates were produced by the coupling of 2,4 dichloropyrimidine with a range of arylboronic acids or esters using Suzuki–Miyaura reaction in dioxane or DMSO.[11] The reactions utilized tetrakis(triphenylphosphine)palladium(0) and 2 M Na_2CO_3 giving intermediates **7**–**12** in yields ranging from 30 to 80%. Compounds **13a**–**q** were produced by the acid catalyzed substituted of the 2-chloropyrimidine of each intermediate with a range of anilines. The typical procedure involved reactions at 90 °C in isopropanol with either TFA or 1 M HCl for up to 10–24 h.

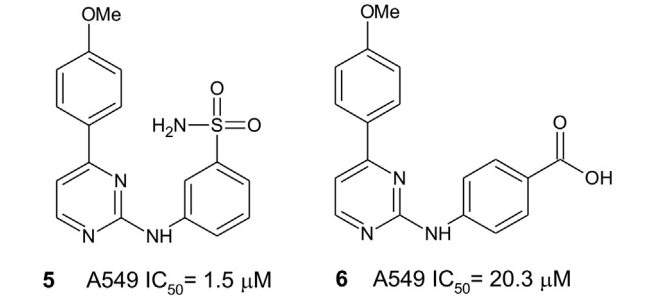

Fig. 2. Initial compounds prepared showing good activity following cytotoxicity assessment in the NSCLC based A549 cell line.

The cytotoxicity of compounds were evaluated in A549 cells (ATCC CCL-185) using an MTT based assay. The compounds displayed IC_{50} values between 0.2 and >100 μM (Table 1). Compounds **13c** and **13f** showed the most potent activity, with IC_{50} of 0.2 μM. This compares to 0.4 μM and 22 μM for the standards Doxorubicin and Gefitinib, respectively. Six different aryl boronic acids groups have been incorporated at the R1 position. Compounds with 3-sulfonamido aniline at the R2, phenyl at the R1 (**13f**) gave rise to the best activity, 8-fold greater than the initial hit **5**, which possesses a 4-methoxy-phenyl. The equivalent 3-methoxy-phenyl analog (**13g**) and *N*-4-acetamido phenyl (**13j**) were over 10-fold less potent than **13f**, while all 4-methylsulphonyl phenyl compounds (**13h** and **13i**) were inactive. Compounds with 2-thiophene-5-Me at R1 showed comparable or slightly weaker activity than the comparable 4-methoxyphenyl compounds (**6** vs **13n** and **13b** vs **13o**). The data collectively points to hydrophobicity being preferred at the R1 for strong A549 activity.

The preference for hydrophobicity at the R1 necessitated greater effort to incorporate polar modifications at R2 in order to maintain a balance in the overall molecular properties (Fig. 3). Compounds with 3-sulfonamido aniline (**5**, **13f**, **13g**, **13j**) displayed the good to reasonable activity. 3-*N*-methylsulphonamido aniline also led to very good activity (**13c**). 4-sulfonamido aniline was over 2-fold less active than the 3-isomer (**5** vs **13e**). 3 and 4-amidobenzamide and amino-benzoic acid were less active than the comparable sulphonamides as were bicyclic amines represented by **13l** and **13m**. The SAR is broadly in-line with that expected for a subset of MAPK kinases, those possessing relatively small hydrophobic back pocket and allow for polar interactions towards the solvent accessible pocket (Fig. 4).

Additional selectivity studies were undertaken using monkey kidney epithelial cells (Vero, ATCC CCL-81)[39,40] using an MTT based approach.[41] Doxorubicin is found to have approximately 5-fold selectivity over A549 cells, and Gefitinib approximately 4.5-fold. The 4-aryl-*N*-phenylpyrimidin-2-amines are found to display low levels of selectivity overall. Compounds **6**, **13c**, **13f**, **13k** and **13n** display comparable levels of selectivity to Doxorubicin (~4-fold). Eight of the most potent and/or selective compounds were further evaluated KB (ATCC CCL-17), MCF7 (ATCC HTB-22) and NCI-H186 (ATCC CRL-5804) using a resazurin based cytotoxicity assays (Table 2).[42] Compounds **5** and **13g**, showed good potency at the 3 additional cell lines, similar in overall profile to Doxorubicin. **13f** showed good activity at 2 of the 3 additional cell lines in line. Compounds **6**, **13a**, **13b**, **13c** and **13d** were more selective, showing either weak to negligible inhibition of the additional cell lines.

All compounds lie within druglike property space and it appears a combination of moderate calculated lipophilicity (clogP) and size showed the best A549 activity (Fig. 3). We determined the experimental solubility and the partition coefficient at $pH_{7.4}$ ($logD_{7.4}$), using shake flask based methodologies, to more thoroughly assess their potential as leads (Table 2). Lead-like physicochemical properties are considered $logD_{7.4}$ <4, while acceptable solubility is considered >10 μM.[43] The compounds showed favorable $logD_{7.4}$ values in the range of 1.2–3.0, with an average of 2.7. **5** was the most soluble in phosphate buffer $pH_{7.4}$ (38.4 μM). Compound **6** had ~3-fold lower solubility and remainder had solubility <10 μM.