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Original article

Synthesis and SAR study of acridine, 2-methylquinoline and 2-phenylquinazoline analogues as anti-prion agents

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Abstract

Transmissible spongiform encephalopathies (TSEs) are thought to arise from aggregation of a protease resistant protein denoted PrP^{Sc}, which is a misfolded isoform of the normal cellular prion protein PrP^{C}. Using virtual high-throughput screening we have selected structures analogous to acridine, 2-methyquinoline and 2-phenylquinazoline as potential therapeutic candidates for the treatment of TSEs. From the synthesis and screening of constructed libraries we have shown that an electron-rich aromatic ring attached through an amine linker to the position para to the ring nitrogen is beneficial to both binding to PrP^{C} and the suppression of PrP^{Sc} accumulation for acridine and 2-methylquinoline analogues. 2-Phenylquinazoline analogues appear to utilise a different mode of action by binding at a different location and/or pose. We report IC_{50}s in the nanomolar range.

Keywords: Prion protein; Acridine; Quinoline; Quinazoline; TSE; vCJD

1. Introduction

Transmissible spongiform encephalopathies (TSEs) are fatal disorders of the central nervous system (CNS) that become symptomatic due to the aggregation of misfolded protein affecting cerebral function. In humans these maladies include Creutzfeldt–Jakob disease (CJD), Gerstmann–Straussler–Scheinker disease, and fatal familial insomnia. TSEs in the animal kingdom include bovine spongiform encephalopathy (BSE), scrapie and chronic wasting disease among others. According to the protein-only hypothesis [1] the infectious particle responsible for progression and transmission of TSEs is a partially protease resistant protein known as PrP^{Sc} or PrP-res [2]. Insoluble PrP^{Sc} is susceptible to accumulation forming fibres, which tend to aggregate in the CNS tissue and instigate a degeneration of brain function, leading to progressive dementia or ataxic illness [3,4]. The normal cellular isoform of the prion protein, denoted PrP^{C} or PrP-sen, is intrinsically soluble and sensitive to protease digestion. PrP^{C} is widely distributed throughout mammalian cells as a glycosylated cell-surface protein of apparent importance due to its genetic and structural conservation between species [5]. The function of PrP^{C} is currently undetermined but reports suggest it to be involved in copper transport, metabolism or cell signalling, along with other potential roles [6,7]. Nevertheless, PrP-knockout mice models showed no signs of neurodegeneration or histopathological changes for up to 15 months, endorsing PrP^{C} as a target for therapeutic intervention [8]. The conversion of the prion protein from a soluble protease sensitive form to that of an insoluble protease resistant form occurs concomitantly with a conformation change in the tertiary structure, where regions of α-helicity are converted into β-sheet [9]. The trigger for this change is little understood but most likely involves destabilisation of the native isoform. It has been hypothesised that a 'protein X' might act as a molecular chaperone and aid the conversion [10].

To date, there are no therapies available for the treatment of prion diseases and as such all recorded cases have proven fatal. One possible mode of action for therapeutic candidates is that of binding to PrP^{C} to increase its stability towards conformational change [11]. Quinacrine was shown to be a potent inhibitor of prion disease in cultured neuroblastoma cells during a recent study, which also showed inhibition brought about by lysomotropic agents and cysteine protease inhibitors [12]. Although

Abbreviations: PrP^{C}, normal cellular prion protein or PrP-sen; PrP^{Sc}, disease-causing isoform or PrP-res; SMB, Scrapie-infected mouse brain; SPR, surface plasmon resonance; vCJD, variant Creutzfeldt–Jakob disease; VHTS, virtual high-throughput screening.

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quinacrine inhibits PrP^{Sc} accumulation it is considered an unsuitable drug candidate due to its toxicity [13]. Analogues of acridine, phenothiazine and other tricyclic compounds, including quinacrine, bind to PrP^{C} via specific amino acid residues [14]. High-throughput screening assays have also highlighted a range of potent inhibitors from natural products and known drugs that include analogues of bicyclic- and tricyclic- nitrogen containing aromatic systems such as quinoline, acridine and phenothiazine; some of which display IC_{50}s in the nanomolar range [15]. These prior studies into the activities of phenothiazines, acridines and quinolines have focused almost exclusively on compounds containing aliphatic side chains [16,17].

Using virtual high-throughput screening (VHTS) a library of commercially available drug-like molecules were docked into a hypothetical binding pocket within PrP^{C}, identified from SYBYL-Molcad surfaces generated from the PrP^{C} NMR structures available from the Brookhaven protein data bank (1QM3, conformer 15). Consensus scoring was used to rank chemical structures according to their predicted binding affinity using a list of independent scoring functions. These include LUDI, PLP1&2, PMF, Jain and functions developed for LigandFit (ligScore1 and LigScore2) [18]. A selection of the highest ranked compounds was sourced and screened for binding to $huPrP^{C}$ using a surface plasmon resonance (SPR) binding assay [19]. Within the hit compounds structures **A1**–**A4** were identified as quinacrine-like substances, each consisting of nitrogen containing fused aromatic systems (Fig. 1). A small number of commercially available analogues of these parent compounds that displayed variation in the location and nature of side chains were assayed for binding to hu- and $moPrP^{C}$. The results of this binding study indicated that aromaticity at the side chain para to the ring nitrogen could be of benefit. Using a rational design approach, libraries of analogous compounds were synthesised. Structure–activity relationships relating both to binding to PrP^{C} and inhibition of PrP^{Sc} accumulation were investigated. Acridines, 2-methylquinolines and a further class of potential prion therapeutics based upon 2-phenylquinazoline were varied by substitution of a side chain para to the ring nitrogen. The effects of this change on binding affinity to PrP^{C} and inhibition of PrP^{Sc} accumulation in scrapie-infected mouse brain (SMB) cell lines are reported here.

2. Chemistry

The lead structures selected by analysis of in vitro screening data were analogous to acridine **1**, 2-methylquinoline **2**, and 2-phenylquinazoline **3** (Fig. 2). In all three lead compounds the side chain is attached through an amine, which provided simple synthetic routes involving substitution at a halogenated carbon. Former studies into compounds comparable to those reported here lack detailed SAR studies and focus on aliphatic side chains extending from the 9 and 4 positions of acridine and quinoline, respectively [16,17]. To avoid repetition, the acridine and 2-methylquinoline libraries were based solely on aromatic side chains. For 2-phenylquinazoline, a larger library was constructed encompassing both aliphatic and aromatic moieties. Indeed, our results suggest that aromaticity in these positions is of benefit to binding over aliphatic side chains. Observations as to the effect that the π-system itself has on binding and activity was probed by altering the functional groups attached to the side chain ring.

2.1. Library synthesis

Libraries were synthesised using 9- and 4-chlorinated lead structure where appropriate. 9-Chloroacridine **4**, 4-chloro-2-methylquinoline **5** and 4-chloro-2-phenylquinazoline **6** were commercially available compounds from which analogues were synthesised by reaction of the appropriate amine via S_N2 type reactions.

In most cases, acid catalysis was utilised to activate the chlorine bearing carbon towards nucleophilic attack by the amine. Exceptions to this rule were those pairings of starting materials in which the amine was more basic than the skeletal nitrogen. For library 1, based on analogues of acridine, syntheses were carried out as reported by Sun et al. [20] (Scheme 1). The reactions were acid catalysed at room temperature and followed by TLC until completion. Reaction times for this library were typically 24 hours, although some were complete within 90 min. An exception to this methodology, within the acridine-based library, led to the synthesis of product **1l**. The amine used for synthesis of **1l** was protonated preferentially to 9-chloroacridine under the usual acidic conditions resulting in a failure to produce the desired product. Phenol was utilised as reported by Castello et al. [21] to form a phenolic intermediate

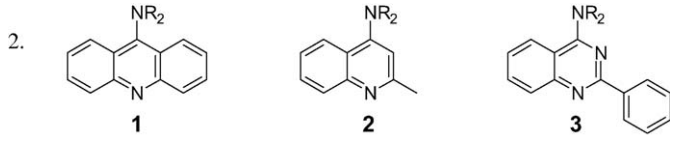


Fig. 2. Acridine, 2-methylquinoline and 2-phenylquinazoline—core structures for the libraries as selected by analysis of VHTS hit compounds.

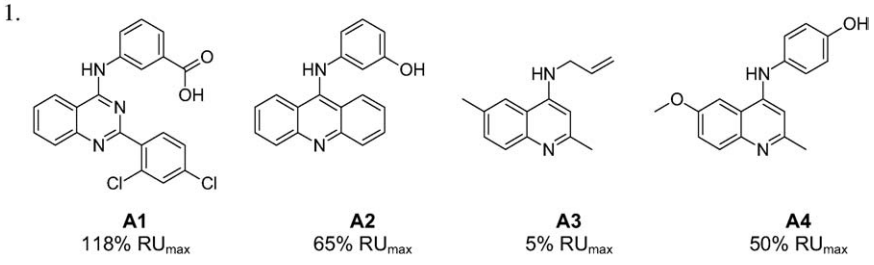
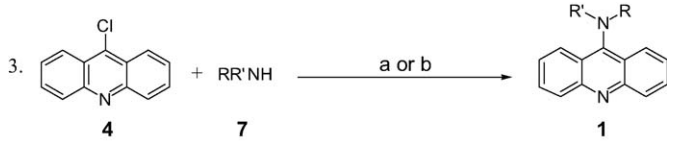


Fig. 1. Hit compounds **A1**–**A4**. Binding toward $huPrP^{C}$ as determined by SPR binding assay.



Scheme 1. Synthesis of an acridine-based library. (a) NMP, HCl (cat.), 25 °C. (b) Phenol 120 °C.

of heightened reactivity relative to 9-chloroacridine. The increased reactivity of this intermediate allowed 6-methoxypyrimidin-4-ylamine **7l** to attack the appropriate carbon centre. The yields obtained during synthesis of the acridine-based library fell between 17% and 89% (Table 1).

The synthesis of library 2, based on 2-methylquinoline, used methodology laid in place by Baig and Stevens (Scheme 2) [22]. The amines utilised in the construction of the library were reacted with 4-chloro-2-methylquinoline in acetone at reflux with an acid catalyst to afford the products as their hydrochloride salts. Reaction times were typically 18 hours but with monitoring by TLC some were deemed complete after as little as 90 min. Yields ranged from 36% to 99% (Table 2). The exception to this methodology was that employed for product **2y** by which 4-chloro-2-methylquinoline was reacted with 6-methoxypyrimidin-4-ylamine **7l** in phenol, allowing synthesis via a phenolic intermediate in a similar manner to compound **1l**. Following characterisation by NMR and ESI-MS the product obtained was shown not to be the desired 4-amino-2-methylquinoline but the phenolic intermediate. The reaction afforded a 55% yield of **8** (Table 2).

The 2-phenylquinazoline-based library was larger than the previous two. Those products derived from aromatic amines were synthesised almost exclusively under acid catalysed conditions in acetone (Scheme 3). Reaction times were typically around 20 hours, although **3b** only required 5 hours reaction time to reach completion. Again, amine 6-methoxypyrimidin-4-ylamine **7l** would not react under these conditions so another route was employed. Syntheses involving aliphatic amines did not favour acid catalysed conditions as the increased basicity of the aliphatic amines meant that they were protonated more readily than **6**, thus preventing activation of the carbon-chlorine bond to nucleophilic attack. An adaptation of a procedure reported by Ananthan et al. [23] in which the reaction takes place in dimethylformamide (DMF) with an excess of amine was employed to avoid using acid catalysis. The reactions were carried out in dioxane for easier solvent removal with a 1:5 ratio of 4-chloro-2-phenylquinazoline to amine. The excess of amine drives the reaction to completion by consuming the HCl released during the reaction and forming its hydrochloride salt. Reaction times ranged from 10 min to 94 hours and yields were typically above 80%. This methodology was applied to the coupling reaction of 6-methoxypyrimidin-4-ylamine **7l** that had been problematic, the reaction reaching completion after 70 hours. Product **3l** was isolated form the mixed crude products by flash column chromatography in a disappointing 3% yield. For the synthesis of **3w**, procedure C was modified to include the basification of the reaction mixture using sodium carbonate. At pH 7 ε-amino-*n*-caproic acid **7w** exists as a zwitterion permitting the amine group to exist in its protonated form RNH_3^+ and thus prohibiting reaction with the electrophile. By basifying the reaction mixture the freed electron pair of the amine could react. Once the reaction was considered complete it was neutralised with acetic acid before being reduced to dryness and the crude product recrystallised from ethyl acetate to yield **3w** in a 90% yield (Table 3).

3. Biological screening

3.1. Binding affinities toward $huPrP^C$ and $moPrP^C$

Binding affinities toward $huPrP^C$ and $moPrP^C$ were determined using a BIAcore 3000, which utilises surface plasmon resonance (SPR) technology. The BIAcore system operates by measuring the real time change in the angle of reflected light upon association of ligand and displays the results as a sensogram. The size of this change is directly proportional to the mass of the immobilised ligand and so the binding affinity was measured as percentage of the maximum response expected for a 1:1 binding ratio with units % RU_{max}. The detection limits of SPR means that the detection of large molecular weight entities is more reliable, such as protein–protein interactions. Detection of small molecule–protein interactions is therefore highly dependant on the level of protein immobilisation. However, a lack of homogeneity in protein immobilisation has led to poor reproducibility between runs. For this reason compounds were initially analysed by triplicate injection. All binding compounds were then screened in one run (single injection only) to allow direct comparison of quantitative binding data. The proteins under discussion were bound to the carboxymethylated dextran surface of a sensor chip and specific concentrations of ligands (typically 40 μM) were passed over the chip in phosphate buffer (6.5% DMSO, 150 mM NaCl, 3 mM EDTA, 0.005% surfactant P20). The binding ligands then dissociate in the continued stream of phosphate buffer.

3.2. SMB cell line screening

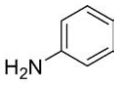
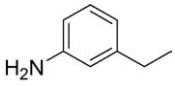
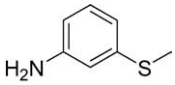
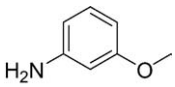
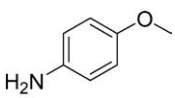
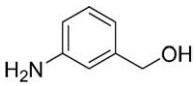
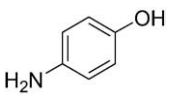
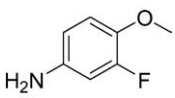
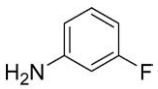
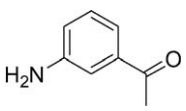
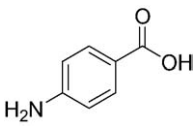
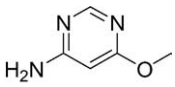
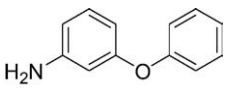
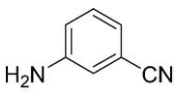
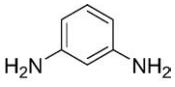
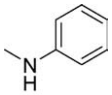
The compounds were assessed for activity and toxicity in SMB cell lines. The dosing of cells was carried out in duplicate with one experiment utilised for toxicity studies and the remaining cells assessed for activity. The viability of cells and the concentration of PrP^{Sc} per experiment were measured as a percentage of untreated controls. Pentosan polysulphate (PPS) at 100 ng ml^{-1} acted as a positive control. Compounds are deemed toxic if the viability of cells falls below 70% of the untreated control. Compounds were deemed active if PrP^{Sc} levels fall below 70% of the untreated control.

4. Results and discussion

The results of BIAcore screening and SMB cell line assays were evaluated in relation to a compound's structure to investigate the significance these changes might have to binding and/or activity (Tables 1–3).

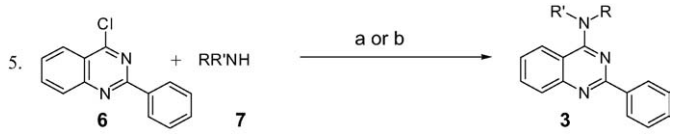
To allow direct comparison of the backbone structures **1**–**3**, 10 amines were used in the construction of each of the three

Table 1
Synthesis and binding data for an acridine based library

Amine		Product	Method [a]	Yield (%)	Binding (% RU_{max})	
					$huPrP^C$	$moPrP^C$
	**7a**	**1a**	A	88	60	16
	**7b**	**1b**	A	59	84	23
	**7c**	**1c**	A	17	151	93
	**7d**	**1d**	A	31	62	19
	**7e**	**1e**	A	37	82	42
	**7f**	**1f**	A	42	71	53
	**7g**	**1g**	A	46	159	130
	**7h**	**1h**	A	89	69	30
	**7i**	**1i**	A	79	59	14
	**7j**	**1j**	A	85	81	43
	**7k**	**1k**	A	18	19	0
	**7l**	**1l**	B	14	20	0
	**7m**	**1m**	A	31	86	107
	**7n**	**1n**	A	83	62	29
	**7o**	**1o**	A	73	336	326
	**7x**	**1x**	A	61	32	1

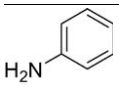
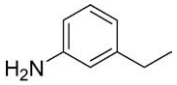
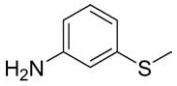
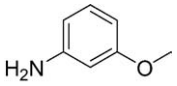
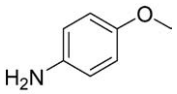
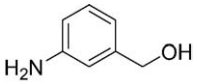
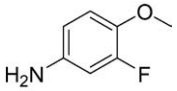
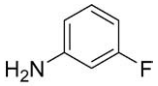
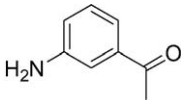
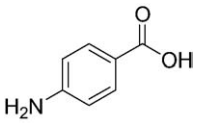
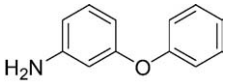
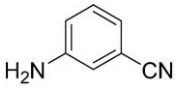
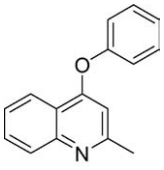
[a] Synthetic method. Refer to experimental section.

Scheme 2. Synthesis of a 2-methylquinoline-based library. (a) Acetone, HCl (cat.), reflux. (b) NMP, HCl (cat.), 25 °C. (c) Phenol, 120 °C.



Scheme 3. Synthesis of a 2-phenylquinazoline-based library. (a) Acetone, HCl (cat.), reflux. (b) Dioxane, reflux.

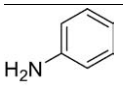
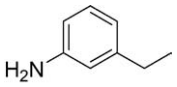
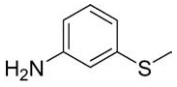
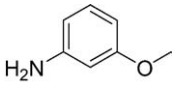
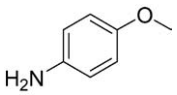
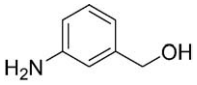
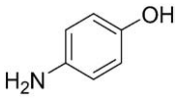
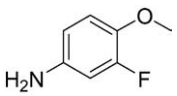
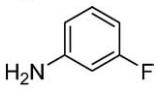
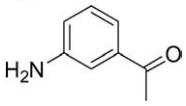
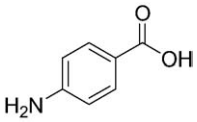
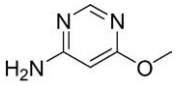
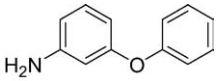
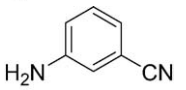
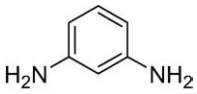
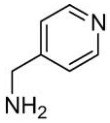
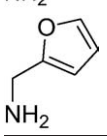
Table 2
Synthesis and binding data for a 2-methylquinoline based library

Amine		Product	Method [a]	Yield (%)	Binding (% RU_{max})	
					$huPrP^C$	$moPrP^C$
	**7a**	**2a**	C	85	37	10
	**7b**	**2b**	C	99	46	14
	**7c**	**2c**	C	68	54	25
	**7d**	**2d**	C	36	51	22
	**7e**	**2e**	C	77	46	17
	**7f**	**2f**	C	74	42	15
	**7h**	**2h**	C	95	33	11
	**7i**	**2i**	C	68	35	12
	**7j**	**2j**	C	82	41	15
	**7k**	**2k**	A	37	23	7
	**7m**	**2m**	C	61	78	29
	**7n**	**2n**	C	46	36	12
		**8**	B	55	24	1

Structure

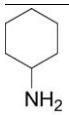
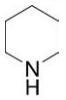
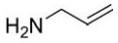
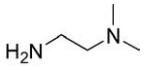
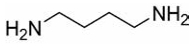
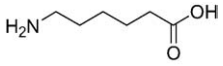
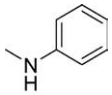
[a] Synthetic method. Refer to experimental section.

Table 3
Synthesis and binding data for a 2-phenylquinazoline based library

Amine		Product	Method [a]	Yield (%)	Binding (% RU_{max})	
					$huPrP^C$	$moPrP^C$
	**7a**	**3a**	C	80	58	37
	**7b**	**3b**	C	92	48	56
	**7c**	**3c**	C	78	56	52
	**7d**	**3d**	C	99	69	50
	**7e**	**3e**	C	86	71	47
	**7f**	**3f**	C	96	– [b]	– [b]
	**7g**	**3g**	C	96	55	36
	**7h**	**3h**	C	99	62	45
	**7i**	**3i**	C	89	67	51
	**7j**	**3j**	C	89	18	4
	**7k**	**3k**	C	98	35	18
	**7l**	**3l**	D	3	– [b]	– [b]
	**7m**	**3m**	C	70	47	52
	**7n**	**3n**	C	80	– [b]	– [b]
	**7o**	**3o**	C	17	50	26
	**7p**	**3p**	D	88	40	16
	**7q**	**3q**	D	84	40	13

(continued)

Table 3 *(continued)*

Amine		Product	Method [a]	Yield (%)	Binding (% RU_{max})	
					$huPrP^C$	$moPrP^C$
	**7r**	**3r**	D	86	21	0
	**7s**	**3s**	D	84	33	2
	**7t**	**3t**	D	90	32	0
	**7u**	**3u**	D	83	21	0
	**7v**	**3v**	D	61	45	17
	**7w**	**3w**	D	90	23	2
	**7x**	**3x**	C	87	57	43

[a] Synthetic method. Refer to experimental section.
[b] Immeasurable result. Interaction with flow cell 1.

libraries. For analysis, the BIAcore data for the 30 compounds is ranked in descending order of binding scores for the 2-phenylquinazoline library (Fig. 3). The libraries of acridine and 2-methylquinoline analogues show similar trends as R is varied. In the majority of cases, a variation that is beneficial to binding in the acridines is also beneficial to the 2-methylquinolines. An example might be the difference in binding affinity observed toward $huPrP^C$ between derivatives of amine **7m** and **7k**, resulting in a decrease in binding. Conversely, changing the amine substituent from **7k** to **7j** increases binding in both cases. No such pattern is observed within the 2-phenylquinazoline as the same amine substituents would be ranked **7m** > **7k** > **7j** in terms of their effect on binding of the 2-phenyquinazolines. Although this is an isolated example, the trend is displayed more fully for binding to both hu- and $moPrP^C$ and suggests that altering the amine substituents has a different effect on the acridines and 2-methylquinolines than the effect the same alterations have on the 2-phenylquinazolines (Fig. 3). Indeed, the effect of altering the amine substituents in the 2-phenylquinazoline library is not very marked at all, suggesting that this class of compounds might bind to the protein in a different location and/or pose. Following this observation it becomes clear that the libraries must be discussed separately.

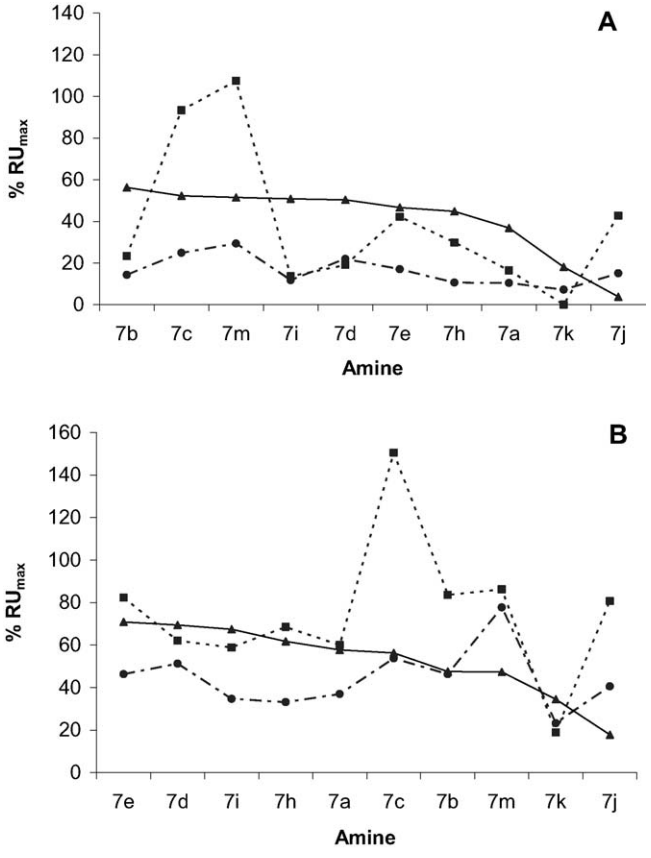


Fig. 3. BIAcore screening results in % RU_{max} for compounds **1, 2** and **3** derived from common amines. **A:** Binding affinities toward $moPrP^C$. **B:** Binding affinities toward $huPrP^C$. In each case scores are ranked in decreasing order of affinity for the 2-phenylquinazolines. Acridines (■) 2-methylquinolines (●) 2-phenylquinazolines (▲).

4.1. Acridines

Our library of acridine-based analogues displays persistently better binding affinities toward hu- and $moPrP^C$ compared with the other libraries discussed here. The majority of binding scores were found to be within the range of 50–100% RU_{max} (Table 1). In exception to this trend compounds **1k** and **1l** displayed binding of approximately 20% RU_{max} towards $huPrP^C$

and did not bind to moPrP^C. These compounds contain electron poor substituents which obviously disfavour the binding of the acridines. In contrast, compounds **1i** and **1j**, which bear electron-withdrawing groups, bind reasonably well to both hu- and moPrP^C. This may be due to the meta position of the electron withdrawing substituents disfavouring formation of a δ-positive charge at the carbon adjacent to the NH linker. Although **1k** houses a meta-methoxy group this seemingly does not overcome the intrinsic electron-poor nature of the pyrimidine ring and also contributes to a δ-positive charge at the aforementioned carbon centre.

Compound **1x** is comparable in structure to **1a**, differing only by the substitution of a methyl group at the nitrogen linker in place of hydrogen. The effect of this change demonstrates the importance of a hydrogen atom at this position since its removal reduces binding dramatically. Additionally, during analysis of the VHTS and in vitro assay hits prior to analogue synthesis, structures containing aliphatic R groups were found not to bind to hu- and moPrP^C. With this in mind, we suggest that an aromatic moiety is essential for binding in this class of compounds and the inclusion of an NH linker is significantly beneficial. Compounds **1b** to **1o** are variations on this relatively simple structure. Compounds in which the π-system of the phenyl ring is made electron deficient with respect to **1a** have comparatively diminished binding affinities. Our data suggests that including groups on the phenyl ring that enrich the electron density of the π-system increase the binding beyond that of **1a**.

4.1.1. Methylquinolines

The library of 2-methylquinoline analogues did not perform as well as the acridines in the BIAcore screening with the majority of results within the range of 30–60% RU_{max} (Table 2). Previous studies surrounding quinoline derivatives as therapeutic candidates for TSEs have focused on aliphatic side chains extending from the position para to the ring nitrogen (in the work by Murakami-Kubo et al. SPR screening was carried out at 100 μM) [17]. In a similar manner to the acridines cluster, the data suggests that aromaticity at this position is of significant benefit to binding. The removal of the NH linker was also shown to be detrimental to binding as seen by comparing **2a** with **2y,** a phenolic derivative (Table 2), whereas the 2-methylquinoline analogues show little sensitivity to the inclusion of electron withdrawing substituents attached to the phenyl ring. Compounds **2h**, **2i**, **2k**, and **2n** also contain electron withdrawing groups, but in comparison to the effect on the acridines, are only marginally detrimental to binding. The inclusion of electron pushing functional groups on the phenyl ring was beneficial to binding and the most dramatic change is seen with compound **2m,** in which the amine-derived substituent is bi-aromatic. However, **2m** was shown, in later kinetics studies, to bind non-specifically to huPrP^C.

4.1.2. Phenylquinazolines

As mentioned, initial analyses suggested that the 2-phenylquinazoline analogues might occupy a different binding region and/or pose to the acridines and 2-methylquinolines. Compounds **3a**–**3o** bear an aromatic side chain and as such display the highest binding (Table 3). Conversely, those compounds with aliphatic substituents (**3r**–**3x**) showed markedly diminished binding. This pattern is well illustrated by comparison of **3a** with **3r,** where binding is reduced from 58% to 21% RU_{max} with only the substitution of R is phenyl for R is cyclohexanyl. The absence of an NH linker brought about no significant change in binding. Comparing **3a** with **3x**, which have an NH and NMe linker, respectively, illustrates this observation, as the binding remains the same. Of the 21 analogues within this library only five compounds show binding higher than that of **3a** including **3l**, which binds non-specifically. These compounds contain R groups with electron withdrawing substituents, electron pushing substituents or even both. **3d, 3e, 3h** and **3i** therefore show no correlation and as such no influential characteristics could be determined.

Analysis of the results from the BIAcore screening and the subsequent SAR study has demonstrated that altering the substituents of the aromatic amine substituent does little to enhance the binding with respect to phenylamine. Together with the observation that the NH linker is not essential in the library of 2-phenylquinazoline series, these analyses support the hypothesis that their binding region and/or pose are different. Whether this effect is due to the additional nitrogen atom in the structure of **6** or the non-fused phenyl group is yet to be shown.

Further to the compounds discussed here a small group of 4-aminopyridine analogues were also synthesised and screened but displayed no binding towards hu- or moPrP^C and no antiprion activity in cell lines (data not shown). This observation suggests that at least two fused aromatic rings are necessary for biological activity as shown by the increased activity of 2-methylquinoline analogues.

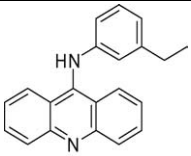
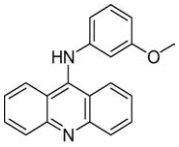
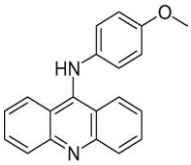
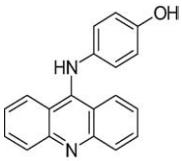
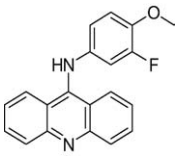
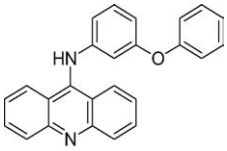
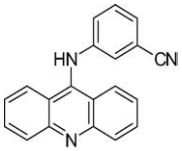
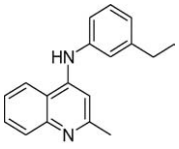
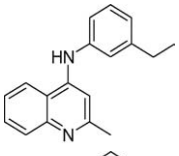
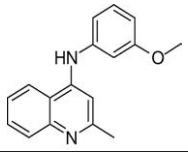
4.2. Inhibition of PrP^{Sc} formation

Each of the drug candidates was tested for activity in SMB cell lines by quantifying their ability to suppress PrP^{Sc} formation relative to an untreated control. For each experiment the level of total cell protein was determined along with the viability of the cells relative to an untreated control. If a compound was seen to affect the viability of the cells they were classed as cytotoxic and rescreened at lower concentrations. This was to ensure that a reduction in PrP^{Sc} levels could only be attributed to the activity of the compounds and not a reduction in cell number. The parents of the acridine and 2-methyquinoline libraries displayed activity at 1 μM whereas the 2-phenylquinazoline parent **A1** was inactive at 10 μM. The lowest concentration at which any parent displayed cytotoxicity was at 10 μM. IC_{50}s of parents **A2**–**A4** were within the range of 1–5 μM without exception (data not shown).

Following the screening of the parents, the libraries of compounds were initially screened at 8 μM for toxicity and activity, a concentration below that at which cytotoxicity was first observed for the parents. Compounds that showed no activity or toxicity were rescreened at the same concentration for confirmation. The cytotoxicity and activity of those compounds

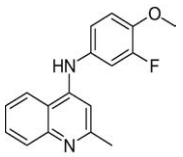
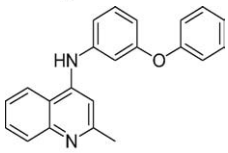
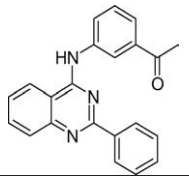
showing a reduction in cell viability at 8 μM were reassessed at lower concentrations. Those compounds that showed activity without toxicity at 8 μM were either rescreened at an appropriate concentration range to determine approximate IC_{50}s or IC_{50} ranges (Table 4), or confirmed active by rescreening at either the same concentration (where standard deviations are shown)

Table 4
SMB cell line screening data for compounds that displayed activity. Cell viability is > 70% of the untreated control at the active concentrations presented

Entry	Structure	Conc. (μM)	PrP^{Sc} level (%) [a]	Toxic conc. (μM) [b]	IC_{50} range (μM) [c]
1b		8 (10)	24 (63)	15	8.0–10.0
1d		1	64 ± 9	8	> 1.0
1e		1	48 ± 5	2	~ 1.0
1g		1	40 ± 11	8	≤ 1.0
1h		0.25	57 ± 9	1	~ 0.25
1m		2.5 (5)	62 (18)	10	2.5–5.0
1n		2.5 (5)	32 (23)	15	1.0–2.5
2b		2.5	64 ± 13	> 10	2.5–5.0
2d		10	53 ± 10	15	~ 10.0
2h		7.5 (8)	51 (48)	15	~ 7.5

(continued)

Table 4 *(continued)*

Entry	Structure	Conc. (μM)	PrP^{Sc} level (%) [a]	Toxic conc. (μM) [b]	IC_{50} range (μM) [c]
2m		0.5	52	5	~ 0.5
3j		2.5	57 ± 8	> 15	~ 2.5
3e		5	65 ± 2	8	5.0–8.0

[a] Level of PrP^{Sc} relative to untreated control present at the stated concentration.
[b] Concentration at which cells are < 70% viable relative to controls.
[c] IC_{50}, approximate concentration giving 50% inhibition of PrP^{Sc} formation.

or at another concentration (shown in brackets). Compounds without activity were omitted from the Table for brevity and thus any compound not included was considered inactive.

An initial observation was that compound **1h**, a tri-aromatic ring structure displayed increased activity above compound **2h**, containing a bi-aromatic ring system. Further to the three libraries discussed a small group of 4-aminopyridine analogues, including a structure derived from amine **7h,** were screened and displayed no binding or activity (data not shown). These observations suggest that while reducing the aromatic system from 3 to 2 fused rings might be detrimental to activity, reducing the system to one ring removes all anti-prion activity.

In the case of all three libraries the NH linker is seemingly essential, compounds **1x**, **3x** and **8** displaying no activity. The 2-methylquinoline and acridine libraries contain the majority of the active compounds discussed herein and share many similarities. Each of the amine derived side chains that provoke activity in the 2-methylquinoline library do so within the acridine library. In general, side groups with oxygen containing, electron pushing substituents promote the activity of these classes of compounds. Whether these groups act as hydrogen bond acceptors or if the effect of the substituents is on the electronic nature of the aromatic ring is yet to be seen. As discussed previously, these functional groups also contribute to the binding of the acridines and 2-methylquinolines and so data suggests there to be a relationship between binding and activity. Contrary to this observation, acridine analogue **1n** contains an electron withdrawing substituent on the aromatic side group and yet displays activity. The cyano functionality of **1n** appears at the meta position relative to the NH linker and so the activity might occur because, although electrons are withdrawn from the aromatic ring, the δ-positive charge is directed away from the carbon adjacent to the linker avoiding electron deficiency where electron density was shown to be important to binding. Alternatively, the cyano group might be metabolised by the cell.

The phenylquinazoline library clearly shows no such correlation of activity with the acridine or 2-methylquinoline libraries. Just two compounds, **3j** and **3e**, show activity below cytotoxic concentrations (Table 4). Compound **3j** houses a strongly electron withdrawing group unseen in any other active compounds save for compound **1n** discussed previously. Again, the ketone group might be metabolised by the cell before reaching the site of action, but nevertheless, data strongly suggest that factors which control the activity of the 2-phenylquinazoline library might differ from those that control the activity of the acridine and 2-methylquinoline libraries. It appears that electron-donating groups do not have any control over activity in this class and this observation strengthens the suggestion that these compounds utilise a different mode of action against prion protein accumulation. Compounds within the 2-phenylquinazoline library are more cytotoxic than the other libraries containing the only compounds showing toxicity at 1 μM, **3d** and **3w** (data not shown). Worth noting is the toxicity of **3d** which includes a functional group that promotes activity in libraries **1** and **2**.

The majority of the compounds discussed herein are toxic at concentrations of 15 μM or below. Bearing in mind that many of the compounds are also active within the range of 1–15 μM it becomes clear that the activity threshold for many compounds is extremely close to that for cytotoxicity. For instance, compound **1e** displays activity at 1 μM but when the concentration is increased to 2 μM the cell viability begins to be affected. An example of an IC_{50} curve is shown for compound **2b** (Fig. 4). The toxicity of this compound is above 10 μM and so is a clear example of a compound whose activity increases in a concentration-dependant manner. Similar curves for **1h**, **1m** and **1n** were constructed and although their IC_{50}s were judged to be below 5 μM, they displayed an increase in PrP^{Sc} levels when nearing their cytotoxic concentrations. It is possi-

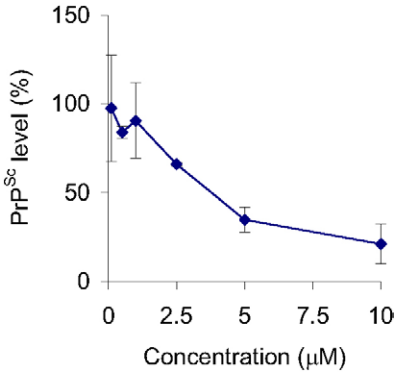


Fig. 4. Dose–response curve for compound **2b**. Compound affects cell viability at concentrations > 10 μM.

ble that this increase in PrP^{Sc} is due to effects on cell viability, in that as the cell encounters marginally toxic concentrations of these compounds the levels of PrP^{C} increases as part of a cellular stress response. As a result of this behaviour, it is possible that some compounds may be active at concentrations very close to those at which cell viability is affected, and therefore some compounds may have been deemed inactive in the initial screen when they may in fact have some underlying activity at lower concentrations. This might also explain the anomalous result for the seemingly reduced activity of **1b** at 10 μM (Table 4). As the compound has displayed activity at two concentrations its action against PrP^{Sc} accumulation cannot be disputed.

The active compounds reported by Korth et al. [16] and Murakami-Kubo et al. [17] were tested in neuroblastoma cells. However, there is evidence that it is harder to inhibit PrP^{Sc} formation in SMB cells than in neuroblastoma lines. Pentosan polysulphate was reported to have an IC_{50} of 1.5×10^{-7} g ml^{-1} in SMB cells [24], whilst Priola and Caughey reported an IC_{50} of 1×10^{-9} g ml^{-1} in ScMNB cells [25]. An EC_{50} of 300 nM has been determined for quinacrine in ScN2a cells [16], whilst in SMB cells we have found the IC_{50} to be two orders of magnitude higher [26]. If this trend is general, it can be assumed that the IC_{50} values of the active compounds reported here could be two orders of magnitude lower in neuroblastoma cells. When applied to compounds such as **1h** and **2m** (Table 4) this gives hypothetical IC_{50}s of less than 10 nM, which are quite comparable with the IC_{50}s of QCQH (7.5 nM), PCQH (4 nM) and 2,2′-biquinoline (3 nM) reported in ScNB cells [17].

5. Conclusion

Within this study we have synthesised three libraries based upon lead structures determined by VHTS. The work has focused mainly on aromatic side chains extending from the carbon para to the ring nitrogen of acridine and 2-methylquinoline and a comparable location in a previously unreported potential prion therapeutic 2-phenylquinazoline. We have shown that binding to PrP^{C} is affected by electron inducing substituents attached to this aromatic moiety for the acridine and 2-methylquinoline libraries to their benefit. From the SAR studies discussed we also conclude that the 2-phenylquinazoline analogues may bind to $huPrP^{C}$ in a different location and/or pose to the acridines and 2-methylquinolines. It follows that these compounds may therefore involve a different mode of action against PrP^{Sc} accumulation. This postulation was strengthened following activity studies in SMB cell lines, which demonstrated that factors influencing the activity of the acridines and 2-methylquinolines do not bring about the same beneficial impact to the 2-phenylquinazoline library. The alteration of aromatic side groups attached to structures **1**, **2** and **3** has brought about significant improvements to the parent structure activities. Furthermore, we have discussed the possibility that at least two fused aromatic rings are necessary for binding to PrP^{C} and also anti-prion activity. Reducing the core structure from a bicyclic system to a pyridine ring is detrimental to the biological properties, whereas a tricyclic system, as found in acridine, may be of benefit.

We have formed a solid argument for the substitution of aromatic side groups to increase the binding affinity and drug-like potency of these types of compounds. We have seen that the NH linker itself is likely to be a significant functional group at the position para to the ring nitrogen. Further investigation into the hypothesis that having an electron-rich aromatic group attached to the NH linker at this position would be most beneficial and may lead to the development of increasingly potent potential TSE therapies.

6. Experimental

Melting points were measured using a Bibby-Sterilin SMP10 melting point apparatus. Accurate mass and nominal mass measurements were measured using a Waters-Micromass LCT electrospray mass spectrometer. Infra red spectra were recorded using neat compounds on a Perkin-Elmer Spectrum RX1 FT-IR system equipped with a DuraSampl*IR* II™ diamond ATR solid sample unit, measuring from 4000 to 400 cm^{-1}.

Thin layer chromatography (TLC) was performed using glass backed silica gel 60 plates (0.25 mm layer), and the ascending technique was used with a variety of solvents. Visualisation was achieved using irradiation at either 254 or 365 nm. Flash column chromatography was carried out using Fluorochem silica gel 60 Å. Dry solvents, when required, were obtained from an in house Grubbs solvent purification system.

^{1}H NMR and ^{13}C NMR spectra were recorded (unless otherwise specified) in deuterio-chloroform in 5 mm tubes on a Bruker AC-250 machine at 250 MHz and 62.5 MHz, respectively. Chemical shifts are quoted in ppm downfield with respect to trimethylsilane ($\delta = 0.00$ ppm). Coupling constants (J) are quoted in Hertz to the nearest 0.1 Hz. Compounds screened were > 95% pure by NMR.

6.1. General procedures

A. Aryl chloride **4**, **5** or **6** (0.5 mmol) and aryl amine **7** (1.0 mmol) were dissolved in 1-methyl-2-pyrrolidinone (NMP) containing one drop of concentrated hydrochloric acid

and stirred at room temperature. The reaction was monitored by TLC and when complete the mixture was poured into ethyl acetate (100 ml). The resulting precipitate was collected by suction filtration. The solids were dissolved in hot methanol and poured into ethyl acetate (100 ml) to re-precipitate. The solids formed were collected by suction filtration and dried under vacuum to yield the product as its hydrochloride salt.

B. To aryl chloride **4** or **5** (0.3 mmol) in phenol (0.6 g) was added 4-amino-6-methoxypyrimidine **7l** (0.3 mmol). After stirring at 120 °C for 2 hours the mixture was poured into acetone (100 ml). No precipitation was observed and the acetone phase was separated between aq 1 M HCl solution (100 ml) and ethyl acetate (100 ml). The pH of the aqueous layer was adjusted to 8 by addition of solid $NaHCO_3$ and extracted with DCM (2×50 ml). The combined DCM layers were dried over $MgSO_4$ and concentrated under reduced pressure to yield the product as a solid.

C. Aryl chloride **5** or **6** (0.5 mmol) and aryl amine **7** (1.0 mmol) were refluxed in acetone (11 ml), containing five drops of concentrated hydrochloric acid and followed to completion by TLC. The solids formed were collected by suction filtration and washed with acetone to yield the product as its hydrochloride salt.

D. To aryl chloride **6** (0.5 mmol) in dioxane (3 ml) was added amine **7** (2.50 mmol). The reaction mixture was heated at reflux to completion as determined by TLC. The reaction mixture was taken up in ethyl acetate (50 ml) and washed with water (3×50 ml). The organic phase was dried over $MgSO_4$ and then reduced under vacuum to yield the solid product.

6.1.1. Acridines

*6.1.1.1. 9-Phenylaminoacridinium **(1a)**.* Procedure **A**. Aniline **7a** (106.0 mg, 0.50 mmol), **4** (50.7 mg, 0.55 mmol), reaction time 1.5 h, gave product **1a** (135 mg, 88%). m.p., > 300 °C; IR (Solid): ν_{max} cm^{-1}, 2765, 1633, 1580, 1550, 1517, 1474, 1440, 1408, 1373, 1343, 1276, 1237 and 1157; ^1H NMR (250 MHz, d^6-DMSO): δ 7.37–7.54 (7H, m, Ar-H), 7.93 (2H, t, $J = 8.0$, Ar-H), 8.10 (2H, d, $J = 8.5$, Ar-H), 8.21 (2H, d, $J = 8.6$, Ar-H); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 114.2, 119.7, 124.1, 125.0, 126.3, 127.8, 130.4, 135.6, 140.6, 141.7, 155.6; ESI-MS *m/z*: 271 ($[M + H]^+$); found 271.1237 ($C_{19}H_{15}N_2$ $[M + H]^+$, requires 271.1235).

*6.1.1.2. Acridin-9-yl-(3-ethylphenyl)-amine **(1b)**.* Conditions were as employed for **1a**. 3-Ethylaniline **b** (152.0 mg, 1.25 mmol), **4** (213.7 mg, 1.00 mmol), reaction time 24 hours gave product **1b** (198.5 mg, 59%) as a solid. m.p., 264 °C; IR (Solid): ν_{max} cm^{-1}, 2864.9, 2729.2, 1634.5, 1581.4, 1559.3, 1524.3, 1477.4, 1464.3, 1438.7, 1415.3, 1376.0, 1345.4, 1262.3, 1175.2, 1158.3, 1114.2, 1033.4; ^1H NMR (250 MHz, d^6-DMSO): δ 1.19 (3H, t, $J = 7.6$, CH_3), 2.65 (2H, q, $J = 7.6$, Ar-H), 7.30 (3H, m, Ar-H), 7.44–7.50 (3H, m, Ar-H), 7.99–8.11 (4H, m, Ar-H), 8.25 (2H, d, $J = 8.6$, Ar-H); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 15.5, 28.0, 106.8, 113.7, 119.2, 121.9, 123.6, 125.8, 126.7, 129.9, 135.2, 140.2, 145.9, 154.6; ESI-MS *m/z:* 299 ($[M + H]^+$); found 299.1552 ($C_{21}H_{19}N_2$ $[M + H]^+$, requires 299.1548).

*6.1.1.3. Acridin-9-yl-(3-methylsulphanylphenyl)-amine **(1c)**.* Conditions were as employed for **1a.** 3-(Methylmercapto)-aniline **c** (146.1 mg, 1.05 mmol), **4** (213 mg, 1.0 mmol), reaction time 4.5 h, gave product **1c**. (59 mg, 17%). m.p., 270 °C (dec); IR (Solid): ν_{max} cm^{-1}, 3460, 2830, 1634, 1570, 1552, 1515, 1461, 1437, 1377, 1343, 1276, 1237, 1161, 1092, 1033; ^1H NMR (250 MHz, d^6-DMSO): δ 2.42 (3H, s, SCH_3), 7.16 (1H, d, $J = 7.3$, Ar-H), 7.25 (1H, d, $J = 7.9$, Ar-H), 7.41 (4H, m, Ar-H), 7.97 (2H, t, $J = 7.6$, Ar-H), 8.14 (2H, d, $J = 8.2$, Ar-H), 8.27 (2H, d, $J = 8.5$, Ar-H); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 14.5, 113.9, 119.2, 120.4, 121.1, 123.8, 124.2 125.8, 130.1, 135.2, 140.1, 140.3, 141.8, 155.0; ESI-MS *m/z:* 317 ($[M + H]^+$); found 317.1112 ($C_{20}H_{17}N_2S$ $[M + H]^+$, requires 317.1112).

*6.1.1.4. Acridin-9-yl-(3-methoxyphenyl)-amine **(1d)**.* Conditions were as employed for **1a**. *m*-Anisidine **d** (181.9 mg, 1.48 mmol), **4** (213.7 mg, 1.00 mmol), reaction time 24 hours gave product **1d** (103.3 mg, 31%) as a solid. m.p., 170 °C; IR (Solid): ν_{max} cm^{-1}, 3307.4, 3100.3, 2884.6, 2836.1, 1634.4, 1605.1, 1583.1, 1555.8, 1523.9, 1476.4, 1464.8, 1441.2, 1263.9, 1154.9, 1031.2; ^1H NMR (250 MHz, CD_3OD): δ 3.34 (1H, s, NH), 3.80 (3H, s, CH_3), 6.98–7.06 (3H, m, Ar-H), 7.42–7.48 (3H, m, Ar-H), 7.91–8.03 (4H, m, Ar-H), 8.21 (2H, d, $J = 8.9$, Ar-H); ^{13}C NMR (62.5 MHz, CD_3OD): δ 56.1, 111.75, 114.9, 115.1, 118.0, 120.2, 125.3, 126.9, 132.2, 136.9, 141.7, 162.7; ESI-MS *m/z:* 301 ($[M + H]^+$); found 301.1334 ($C_{20}H_{17}N_2O$ $[M + H]^+$, requires 301.1341).

*6.1.1.5. Acridin-9-yl-(4-methoxyphenyl)-amine **(1e)**.* Conditions were as employed for **1a**. *p*-Anisidine **e** (154.9 mg, 1.45 mmol), **4** (213.7 mg, 1.00 mmol), reaction time 24 hours gave product **1e** (125.3 mg, 37%) as a solid. m.p., 284 °C; IR (Solid): ν_{max} cm^{-1}, 3463.7, 2894.5, 2838.3, 1633.2, 1584.7, 1555.5, 1524.0, 1505.7, 1475.9, 1464.7, 1441.6, 1297.5, 1241.8, 1162.1, 1106.2; ^1H NMR (250 MHz, d^6-DMSO): δ 3.87 (3H, s, CH_3), 7.12 (2H, d, $J = 6.7$, Ar-H), 7.43–7.50 (4H, m, Ar-H), 7.98–8.09 (4H, m, Ar-H), 8.25 (2H, d, $J = 8.5$, Ar-H); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 55.5, 113.2, 115.2, 119.1, 123.5, 125.7, 126.4, 125.1, 133.3, 140.0, 155.3, 158.5; ESI-MS *m/z:* 301 ($[M + H]^+$); found 301.1349 ($C_{20}H_{17}N_2O$ $[M + H]^+$, requires 301.1341).

*6.1.1.6. [3-(Acridin-9-ylamino)-phenyl]-methanol **(1f)**.* Conditions were as employed for **1a**. 3-Aminobenzylalcohol **f** (138.6 mg, 1.13 mmol), **4** (213.7 mg, 1.00 mmol), reaction time 24 hours gave product **1f** (142.5 mg, 42%) as a solid. m.p., 255 °C; IR (Solid): ν_{max} cm^{-1}, 3341.9, 2860.0, 2800.1, 1633.6, 1578.3, 1554.0, 1523.2, 1464.1, 1447.3, 1373.6, 1343.6, 1280.0, 1255.4, 1164.1, 1113.6, 1051.7; ^1H NMR (250 MHz, d^6-DMSO): 4.59 (2H, s, CH_2), 7.31 (1H, d, $J = 7.6$, Ar-H), 7.38–7.5 (5H, m, Ar-H), 8.00–8.11 (4H, m, Ar-H), 8.26 (2H, d, $J = 8.9$, Ar-H); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 62.3, 113.7, 122.3, 122.7, 123.7, 125.8, 126.8,

129.6, 135.3, 140.1, 141.2, 144.9, 154.9; ESI-MS *m/z:* 301 ($[M + H]^+$); found 301.1331 ($C_{20}H_{17}N_2O$ $[M + H]^+$, requires 301.1341).

*6.1.1.7. 4-(Acridin-9-ylamino)-phenol (**1g**).* Conditions were as employed for **1a**. 4-Aminophenol **g** (108.9 mg, 1.00 mmol), **4** (213.7 mg, 1.00 mmol), reaction time 24 hours gave product **1g** (149.9 mg, 46%) as a solid. m.p., > 300 °C; IR (Solid): ν_{max} cm^{-1}, 3101.7, 2929.5, 1632.9, 1587.0, 1560.7, 1511.1, 1472.2, 1438.2, 1415.5, 1365.3, 1345.3, 1265.0, 1225.7, 1165.7, 1154.2, 1099.7; ^1H NMR (250 MHz, d^6-DMSO): δ 6.96 (2H, d, $J = 8.9$, Ar-H), 7.31 (2H, d, $J = 8.9$, Ar-H), 7.40 (2H, t, $J = 7.6$, Ar-H), 7.98 (2H, t, $J = 7.6$, Ar-H), 8.09 (2H, d, $J = 8.5$, Ar-H), 8.26 (2H, d, $J = 8.9$, Ar-H), 10.11 (1H, s, NH); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 113.0, 116.5, 119.0, 123.4, 125.7, 126.6, 131.4, 135.0, 140.0, 155.3, 157.2; ESI-MS *m/z:* 287 ($[M + H]^+$); found 287.1186 ($C_{19}H_{15}N_2O$ $[M + H]^+$, requires 287.1184).

*6.1.1.8. Acridin-9-yl-(3-fluoro-4-methoxyphenyl)-amine (**1h**).* Conditions were as employed for **1a**. 3-Fluoro-4-methoxy aniline **h** (143 mg, 1.0 mmol) was reacted with **4** (213 mg, 1.0 mmol) in the presence of acid, reaction time 3.5 h, to yield product **1h** as a solid. (314 mg, 89%). m.p., 257–259 °C; IR (Solid): ν_{max} cm^{-1}, 3461, 2890, 2938, 1636, 1584, 1557, 1509, 1466, 1441, 1379, 1312, 1265, 1226, 1174, 1135, 1103, 1025 and 970; ^1H NMR (250 MHz, d^6-DMSO): δ 2.42 (3H, s, OCH_3), 7.16 (1H, d, $J = 7.3$, Ar-H), 7.25 (1H, d, $J = 7.9$, Ar-H), 7.41 (4H, m, Ar-H), 7.97 (2H, t, $J = 7.6$, Ar-H), 8.14 (2H, d, $J = 8.2$, Ar-H), 8.27 (2H, d, $J = 8.5$, Ar-H); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 56.7, 113.4, 113.7, 113.9, 114.9, 118.9, 119.6, 121.8, 124.2, 126.2, 133.8, 134.0, 135.6, 140.5, 146.8, 147.0, 149.9, 153.8, 155.7; ESI-MS *m/z:* 319 ($[M + H]^+$); found 319.1247 ($C_{20}H_{16}FN_2O$ $[M + H]^+$, requires 319.1258).

*6.1.1.9. Acridin-9-yl-(3-fluorophenyl)-amine (**1i**).* Conditions were as employed for **1a**. 3-Fluroaniline **i** (145.2 mg, 1.31 mmol), **4** (213.7 mg, 1.00 mmol), reaction time 24 h, gave product **1i** (256.1 mg, 79%) as a solid. m.p., 288 °C; IR (Solid): ν_{max} cm^{-1}, 2766.9, 1632.2, 1580.1, 1551.5, 1520.2, 1475.3, 1434.0, 1410.5, 1376.9, 1341.8, 1259.9, 1162.4, 1109.4, 1080.1, 1033.7; ^1H NMR (250 MHz, d^6-DMSO): δ 7.23–7.40 (3H, m, Ar-H), 7.50–7.58 (3H, m, Ar-H), 8.06 (2H, t, $J = 7.6$ Hz, Ar-H), 8.21 (2H, d, $J = 8.6$, Ar-H), 8.32 (2H, d, $J = 8.5$, Ar-H); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 106.8, 106.9, 111.4, 114.3, 119.4, 120.1, 124.1, 125.8, 131.5, 135.4, 140.2, 155.1; ESI-MS *m/z:* 289 ($[M + H]^+$); found 289.1141 ($C_{19}H_{14}FN_2$ $[M + H]^+$, requires 289.1141).

*6.1.1.10. 1-[3-(Acridin-9-ylamino)-phenyl]-ethanone (**1j**).* Conditions were as employed for **1a**. 3-Aminoacetophenone **j** (134 mg, 1.0 mmol) was reacted with **4** (212 mg, 1.0 mmol), reaction time 1.5 h, gave **1j** as a solid. (293 mg, 85%). m.p., 292–294 °C; IR (Solid): ν_{max} cm^{-1}, 2705, 1676, 1636, 1578, 1559, 1519, 1484, 1471, 1414, 1379, 1360, 1316, 1301, 1268, 1226, 1164; ^1H NMR (250 MHz, d^4-MeOH): δ 2.60 (3H, s, C(O)CH_3), 7.46 (2H, m, Ar-H), 7.64 (2H, m, Ar-H), 8.01 (6H, m, Ar-H), 8.20 (2H, d, $J = 8.9$, Ar-H); ^{13}C NMR (62.5 MHz, d^4-MeOH): δ 26.9, 115.7, 120.5, 124.9, 125.6, 126.8, 128.6, 129.9, 131.7, 136.9, 140.2, 142.0, 143.1, 147.2, 157.1; ESI-MS *m/z:* 313 ($[M + H]^+$); found 313.1334 ($C_{21}H_{17}N_2O$ $[M + H]^+$, requires 313.1341).

*6.1.1.11. 4-(Acridin-9-ylamino)-benzoic acid (**1k**).* Conditions were as employed for **1a**. 4-Aminobenzoic acid **k** (77.3 mg, 0.55 mmol) was reacted with **4** (106 mg, 0.5 mmol), reaction time 1.5 h, gave **1k** as a solid. (137 mg, 79%). m.p., > 300 °C; IR (Solid): ν_{max} cm^{-1}, 2837, 2539, 1707, 1632, 1578, 1547, 1509, 1474, 1376, 1272, 1236, 1215, 1167, 1116, 1018; ^1H NMR (250 MHz, d^6-DMSO): δ 7.48 (2H, d, $J = 8.2$, Ar-H), 7.50 (2H, t, $J = 7.9$, Ar-H), 7.98 (2H, d, $J = 8.5$, Ar-H), 8.03 (2H, m, Ar-H), 8.22 (2H, d, $J = 8.5$, Ar-H), 8.33 (2H, d, $J = 8.9$, Ar-H) 11.7 (1H, bs, NH), 15.4 (1H, bs, NH); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 115.4, 119.9, 123.4, 124.8, 126.4, 128.6, 131.3, 136.0, 140.7, 146.2, 155.2, 167; ESI-MS *m/z:* 315 ($[M + H]^+$); found 315.1120 ($C_{20}H_{15}N_2O_2$ $[M + H]^+$, requires 315.1134).

*6.1.1.12. Acridin-9-yl-(6-methoxypyrimidin-4-yl)-amine (**1l**).* Procedure **B**. 6-Methoxypyrimidin-4-ylamine **7l** (34.6 mg, 0.28 mmol), **4** (60.9 mg, 0.29 mmol), reaction time 2 h. The solids obtained were subjected to column chromatography (ethyl acetate/chloroform 1:1, $R_f = 0.76$) to yield product **1l** as yellow amorphous solid. (12.3 mg, 14% yield). m.p., 265–266 °C; IR (Solid): ν_{max} cm^{-1}, 2922, 1584, 1552, 1504, 1474, 1396, 1357, 1284, 1233, 1203, 1162 and 1042; ^1H NMR (250 MHz, $CDCl_3$): δ 3.76 (3H, s, OCH_3), 5.48 (1H, bs, NH), 7.19 (1H, s, Ar-H), 7.41 (2H, t, $J = 7.6$, Ar-H), 7.68 (2H, t, $J = 7.3$, Ar-H), 8.05 (2H, d, $J = 8.5$, Ar-H), 8.16 (2H, d, $J = 8.5$, Ar-H), 8.36 (1H, s, Ar-H); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 53.9, 118.9, 124.0, 126.2; ESI-MS *m/z:* 303 ($[M + H]^+$); found 303.1237 ($C_{18}H_{15}N_4O$ $[M + H]^+$, requires 303.1246).

*6.1.1.13. Acridin-9-yl-(3-phenoxyphenyl)-amine (**1m**).* Conditions were as employed for **1a**. 3-Phenoxyaniline **m** (181.6 mg, 0.98 mmol), **4** (213.7 mg, 1.00 mmol), reaction time 24 hours gave **1m** (123.8 mg, 31%) as a solid. m.p., 221 °C; IR (Solid): ν_{max} cm^{-1}, 3421.1, 3081.8, 2883.4, 2833.9, 1631.9, 1588.4, 1578.1, 1548.1, 1513.8, 1488.4, 1474.0, 1444.2, 1434.6, 1411.1, 1376.7, 1345.2, 1262.3, 1214.0, 1214.0, 1171.3, 1150.4, 1112.2; ^1H NMR (250 MHz, d^6-DMSO): δ 7.08–7.10 (4H, m, Ar-H), 7.17 (1H, t, $J = 7.3$, Ar-H), 7.26 (1H, d, $J = 8.6$, Ar-H), 7.40 (2H, t, $J = 7.9$, Ar-H), 7.53–7.60 (3H, m, Ar-H), 8.02–8.13 (4H, m, Ar-H), 8.32 (2H, d, $J = 8.5$, Ar-H); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 114.1, 114.2, 117.3, 118.7, 119.3, 123.8, 124.0, 125.8, 130.1, 131.3, 135.3, 140.1, 155.1, 145.1, 157.6; ESI-MS *m/z:* 363 ($[M + H]^+$); found 363.1491 ($C_{25}H_{19}N_2O$ $[M + H]^+$, requires 363.1497).

*6.1.1.14. 3-(Acridin-9-ylamino)-benzonitrile (**1n**).* Conditions were as employed for **1a**. 3-Aminobenzonitrile **n** (135.7 mg, 1.15 mmol), **4** (213.7 mg, 1.00 mmol), reaction time 24 h,

gave product **1n** (274.6 mg, 83%) as a solid. m.p., 291 °C; IR (Solid): ν_{max} cm^{-1}, 2735.7, 2211.8, 1635.4, 1578.5, 1554.1, 1516.0, 1474.5, 1435.4, 1410.8, 1375.7, 1350.6, 1269.6, 1158.0, 1029.8; ^{1}H NMR (250 MHz, d^6-DMSO): δ 7.55 (2H, t, J = 7.3, Ar-H), 7.72 (2H, d, J = 7.0, Ar-H), 7.84 (1H, d, J = 7.0, Ar-H), 7.92 (1H, s, Ar-H), 8.08–8.13 (4H, m, Ar-H), 8.27 (2H, d, J = 8.9, Ar-H); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 106.5, 112.4, 114.8, 118.2, 119.5, 124.3, 125.8, 126.7, 128.2, 130.6, 131.0 135.5, 140.3, 154.9; ESI-MS *m/z:* 296 ($[M + H]^+$); found 296.1187 ($C_{20}H_{14}N_3$ $[M + H]^+$, requires 296.1188).

*6.1.1.15. N-acridin-9-yl-benzene-1,4-diamine (**1o**)*. Conditions were as employed for **1a**. 1,3-Phenyldiamine **o** (118.8 mg, 1.10 mmol), **4** (213.7 mg, 1.00 mmol), reaction time 24 h, gave product **1o** (236.2 mg, 73%) as a solid. m.p., 266 °C (decomposed); IR (Solid): ν_{max} cm^{-1}, 2791.5, 2586.2, 1633.4, 1577.8, 1552.3, 1517.8, 1485.4, 1472.3, 1434.6, 1412.3, 1375.1, 1347.4, 1266.1, 1156.4, 1114.3, 1032.3; ^{1}H NMR (250 MHz, d^6-DMSO): δ 6.85 (2H, d, J = 7.9, Ar-H), 7.29 (1H, t, J = 7.8, Ar-H), 7.47 (1H, d, J = 9.1, Ar-H), 7.56–7.66 (2H, m, Ar-H), 8.07, (2H, t, J = 7.5, Ar-H), 8.20 (2H, d, J = 8.6, Ar-H), 8.44 (2H, d, J = 8.6, Ar-H); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 106.8, 114.5, 118.9, 122.9, 123.5, 125.8, 130.0, 135.1, 140.2, 155.2; ESI-MS *m/z:* 286 ($[M + H]^+$); found 286.1344 ($C_{17}H_{16}N_2O_2$ $[M + H]^+$, requires 286.1332).

*6.1.1.16. Acridin-9-yl-methylphenyl-amine (**1x**)*. Conditions were as employed for **1a**. *N*-Methylaniline **x** (173.7 mg, 1.62 mmol), **4** (213.7 mg, 1.00 mmol), reaction time 24 hours gave product **1x** (194.9 mg, 61%) as a solid. m.p., 249 °C; IR (Solid): ν_{max} cm^{-1}, 2365.0, 1878.1, 1631.4, 1575.7, 1489.4, 1470.0, 1433.9, 1408.1, 1380.2, 1291.9, 1209.9, 1155.3 and 1100.4; ^{1}H NMR (250 MHz, d^6-DMSO): δ 3.84 (3H, s, CH_3), 6.97–7.01 (3H, m, Ar-H), 7.30 (2H, t, J = 7.8, Ar-H), 7.73 (1H, d, J = 8.5, Ar-H), 7.76 (1H, d, J = 7.6, Ar-H), 8.15–8.22 (4H, m, Ar-H), 8.53 (2H, d, J = 8.9, Ar-H); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 42.2, 106.1, 108.9, 116.4, 120.2, 123.6, 126.0, 127.0, 129.5, 135.5, 148.7; ESI-MS *m/z:* 285 ($[M + H]^+$); found 285.1387 ($C_{20}H_{17}N_2$ $[M + H]^+$, requires 285.1392).

6.1.2. 2-Methylquinolines

*6.1.2.1. (2-Methylquinolin-4-yl)-phenylamine (**2a**)*. Procedure **C**. Aniline **7a** (93 mg, 1.0 mmol), **5** (89 mg, 0.5 mmol), reaction time 16 h, gave **2a** as a solid (102 mg, 85%). Alternatively an identical procedure as that employed for **1a** was used. 7**a** (100 mg, 1.1 mmol), **5** (218 mg, 1.0 mmol), reaction time 1.5 h, gave **2a**, after basic extraction, as a solid (free base). (388 mg, 36%). m.p., 185–187 °C; IR (Solid): ν_{max} cm^{-1}, 3473, 2901, 1637, 1608, 1592, 1555, 1492, 1450, 1370, 1278, 1144, 860, 753, 700, 636 and 524; ^{1}H NMR (250 MHz, d^6-DMSO): δ 2.65 (3H, s, CH_3), 6.73 (1H, s, Ar-H), 7.44–7.65 (4H, m, Ar-H), 7.79 (1H, t, J = 7.5, Ar-H), 8.00–8.12 (2H, m, Ar-H), 8.79 (1H, d, J = 8.5, NH), 10.83 (1H, bs, NH); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 19.9, 99.9, 115.8, 119.7, 123.4, 125.5, 126.5, 127.1, 129.9, 133.7, 137.2, 138.1, 154.1, 154.6; ESI-MS *m/z:* 235 $[M + H]^+$; found 235.1237 ($C_{16}H_{15}N_2$ $[M + H]^+$, requires 235.1235).

*6.1.2.2. (3-Ethylphenyl)-(2-methylquinolin-4-yl)-amine (**2b**)*. Procedure **C.** 3-Ethylaniline 7**b** (250.7 mg, 2.07 mmol), **5** (0.20 ml, 1.00 mmol), reaction time 18 h, gave **2b** as a solid. (96.7 mg, 99%) m.p., 258 °C; IR (Solid): ν_{max} cm^{-1}, 2770.0, 1638.8, 1597.8, 1586.1, 1553.9, 1487.6, 1442.5, 1368.8, 1279.5, 1204.2, 1167.7, 1143.8, and 1045.9; ^{1}H NMR (250 MHz, d^6-DMSO): δ 1.27 (3H, t, J = 7.6, CH_3), 2.64 (3H, s, CH_3), 2.73 (2H, q, J = 7.6, CH_2), 6.71 (1H, s, Ar-H), 7.33 (1H, t, J = 5.8, Ar-H), 7.33 (1H, d, J = 5.2, Ar-H), 7.35 (1H, s, Ar-H), 7.52 (1H, t, J = 8.1, Ar-H), 7.79 (1H, t, J = 6.3, Ar-H), 7.99–8.09 (2H, m, Ar-H), 8.76 (1H, d, J = 8.2, Ar-H), 10.74 (1H, s, NH); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 15.4, 19.8, 28.0, 99.9, 116.1, 119.7, 122.7, 123.4, 124.7, 126.5, 126.8, 129.8, 133.6, 137.2, 138.4, 145.8, 154.2, 154.5; ESI-MS *m/z:* 263 $[M + H]^+$; found 263.1543 ($C_{18}H_{19}N_2$ $[M + H]^+$, requires 263.1548).

*6.1.2.3. (2-Methylquinolin-4-yl)-(3-methylsulphanylphenyl)-amine (**2c**)*. Conditions were as employed for **2b**. 3-(Methylmercapto) aniline **c** (142.2 mg, 1.02 mmol), **5** (94.6 mg, 0.53 mmol), reaction time 18 h, gave **2c** as a solid. (115 mg, 68%). m.p., 160 °C; IR (Solid): ν_{max} cm^{-1}, 1596, 1570, 1485, 1412, 1377, 1343, 1208; ^{1}H NMR (250 MHz, d^6-DMSO): δ 2.65 (3H, s, CH_3), 3.18 (3H, s CH_3), 6.76 (1H, s, Ar-H), 7.27–7.37 (3H, m, Ar-H), 7.50 (1H, dd, J = 9.2, J = 8.3, Ar-H), 7.78 (1H, t, J = 7.6, Ar-H), 7.99–8.11 (2H, m, Ar-H), 8.77 (1H, d, J = 8.5, Ar-H), 10.81 (1H, s, NH); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 14.4, 19.8, 106.8, 116.2, 119.7, 121.6, 122.2, 123.4, 124.6, 127.9, 130.2, 133.7, 137.9, 138.4, 140.3, 154.2, 154.7; ESI-MS *m/z:* 281 $[M + H]^+$; found 281.1108 ($C_{17}H_{17}N_2S$ $[M + H]^+$, requires 281.1112).

*6.1.2.4. (3-Methoxyphenyl)-(2-methylquinolin-4-yl)-amine (**2d**)*. Conditions were as employed for **2b**. *m*-Anisidine **d** (248.1 mg, 2.01 mmol), **5** (0.20 ml, 1.00 mmol), reaction time 18 h, gave product **2d** (108.5 mg, 36%) as a solid. m.p., 237 °C; IR (Solid): ν_{max} cm^{-1}, 2683.7, 1638.2, 1586.9, 1553.7, 1488.6, 1390.1, 1287.9, 1259.9, 1200.6, 1158.5, 1099.0, 1035.5; ^{1}H NMR (250 MHz, d^6-DMSO): δ 2.68 (3H, s, CH_3), 3.85 (3H, s, OCH_3), 6.78 (1H, s, Ar-H), 7.01–7.11 (3H, m, Ar-H), 7.51 (1H, t, J = 7.9, Ar-H), 7.77 (1H, t, J = 6.9, Ar-H), 8.02 (1H, t, J = 5.5, Ar-H), 8.19 (1H, d, J = 8.2, Ar-H), 8.88 (1H, d, J = 7.9, Ar-H), 10.94 (1H, s, NH); ^{13}C NMR (62.5 MHz, d^6-DMSO) 19.7, 55.4, 100.2, 106.8, 111.1, 112.8, 116.2, 117.3, 119.6, 123.6, 126.4, 130.6, 133.5, 138.4, 154.2, 154.5; ESI-MS *m/z:* 265 $[M + H]^+$; found 265.1353 ($C_{17}H_{17}N_2O$ $[M + H]^+$, requires 265.1341).

*6.1.2.5. (4-Methoxyphenyl)-(2-methylquinolin-4-yl)-amine (**2e**)*. Conditions were as employed for **2b**. *p*-Anisidine **e** (246.7 mg, 2.00 mmol), **5** (0.20 ml, 1.00 mmol), reaction time 18 h, gave product **2e** (230.7 mg, 77%) as a solid. m.p., 295 °C; IR

(Solid): ν_{max} cm^{-1}, 2686.0, 1638.4, 1607.5, 1588.6, 1553.6, 1489.2, 1447.5, 1367.8, 1288.2, 1260.4, 1201.4, 1159.0, 1143.3, 1099.5 and 1034.3; ^1H NMR (250 MHz, d^6-DMSO): δ 2.61 (3H, s, CH_3), 3.87 (3H, s, CH_3), 6.57 (1H, s, Ar-H), 7.17 (2H, d, $J = 8.9$ Hz, Ar-H), 7.42 (2H, d, $J = 8.9$ Hz, Ar-H), 7.75–7.82 (1H, m, Ar-H), 8.02 (2H, d, $J = 3.4$ Hz, Ar-H), 8.71 (1H, d, $J = 8.5$ Hz, Ar-H), 10.67 (1H, s, NH); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 19.9, 55.4, 99.6, 115.1, 119.6, 123.3, 126.4, 127.3, 129.6, 133.6, 154.3, 154.9, 158.3; ESI-MS *m/z:* 265 $[M + H]^+$; found 265.1345 ($C_{17}H_{17}N_2O$ $[M + H]^+$, requires 265.1341).

*6.1.2.6. [4-(2-Methylquinolin-4-ylamino)-phenyl]-methanol (**2f**)*. Conditions were as employed for **2b**. 3-Aminobenzylalcohol **f** (246.2 mg, 2.00 mmol), **2** (0.20 ml, 1.00 mmol), reaction time 18 h, gave product **2f** (222.3 mg, 74%) as a solid. m.p., 259 °C; IR (Solid): ν_{max} cm^{-1}, 2789.6, 1640.6, 1601.1, 1584.3, 1556.8, 1446.3, 1365.9, 1279.6, 1207.1, 1169.9, 1140.2 and 1046.3; ^1H NMR (250 MHz, d^6-DMSO): δ 2.65 (3H, s, CH_3), 3.20 (2H, s, CH_2), 6.70 (1H, s, Ar-H), 7.38 (2H, d, $J = 7.6$, Ar-H), 7.51–7.59 (2H, m, Ar-H), 7.76 (1H, t, $J = 7.8$, Ar-H), 8.01 (1H, t, $J = 7.6$, Ar-H), 8.15 (1H, d, $J = 8.2$, Ar-H), 8.83 (1H, d, $J = 7.5$, Ar-H) 10.90 (1H, s, NH); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 19.8, 62.3, 99.8, 116.1, 119.6, 123.2, 123.5, 123.6, 125.1, 126.5, 129.6, 133.6, 137.1, 138.1, 144.7, 154.3, 154.5; ESI-MS *m/z:* 265 $[M + H]^+$; found 265.1329 ($C_{17}H_{17}N_2O$ $[M + H]^+$, requires 265.1341).

*6.1.2.7. (3-Fluoro-4-methoxyphenyl)-(2-methylquinolin-4-yl)-amine (**2h**)*. Conditions were as employed for **2b**. 3-Fluoro-4-methoxy aniline **h** (148 mg, 1.1 mmol), was reacted with **5** (96 mg, 0.5 mmol), reaction time 23 h, gave **2h** as a solid. (208 mg, 95%). m.p., 160 °C (dec); IR (Solid): ν_{max} cm^{-1}, 1605, 1509, 1450, 1371, 1284, 1221, 1162, 1129, 1095, 1018; ^1H NMR (250 MHz, d^6-DMSO): δ 2.64 (3H, s, CH_3), 3.94 (3H, s, OCH_3), 6.67 (1H, s, Ar-H), 7.05–7.50 (4H, m, Ar-H), 7.75 (1H, t, $J = 6.7$, Ar-H), 8.04 (1H, d, $J = 8.6$, Ar-H), 8.05 (1H, t, $J = 9.0$, Ar-H), 8.74 (1H, d, $J = 8.9$, Ar-H), 10.76 (1H, s, NH); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 19.6, 56.2, 99.9, 113.8, 114.2, 114.4, 114.5, 116.0, 119.5, 122.3, 123.6, 126.4, 129.8, 133.5, 138.3, 146.2, 154.5; ESI-MS *m/z:* 283 $[M + H]^+$; found 238.1238 ($C_{17}H_{16}FN_2O$ $[M + H]^+$, requires 283.1247).

*6.1.2.8. (3-Fluoro-phenyl)-(2-methylquinolin-4-yl)-amine (**2i**)*. Conditions were as employed for **2b**. 3-Fluroaniline **2i** (219.5 mg, 1.97 mmol), **5** (0.20 ml, 1.00 mmol), reaction time 18 h, gave product **2i** (195.8 mg, 68%) as a solid. m.p., 190 °C; IR (Solid): ν_{max} cm^{-1}, 2901.1, 1638.4, 1591.2, 1556.2, 1487.5, 1442.8, 1385.7, 1370.0, 1256.3, 1207.5, 1141.8, 1099.4; ^1H NMR (250 MHz, d^6-DMSO): δ 2.70 (3H, s, CH_3), 6.88 (1H, s, Ar-H), 7.30 (1H, t, $J = 7.3$, Ar-H), 7.43 (1H, d, $J = 8.6$, Ar-H), 7.48 (1H, s, Ar-H), 7.66 (1H, d, $J = 7.0$, Ar-H), 7.76 (1H, t, $J = 7.3$, Ar-H), 8.04 (1H, t, $J = 7.3$, Ar-H), 8.18 (1H, d, $J = 8.5$, Ar-H), 8.88 (1H, d, $J = 8.2$, Ar-H), 11.01 (1H, s, NH); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 19.7, 100.5, 112.2, 112.6, 113.7, 114.0, 116.3, 119.7, 121.6, 123.6, 126.6, 131.4, 131.5, 138.4, 139.1, 153.9, 154.9, 164.5; ESI-MS *m/z:* 253 $[M + H]^+$; found 253.1145 ($C_{16}H_{14}FN_2$ $[M + H]^+$, requires 253.1141).

*6.1.2.9. 1-[3-(2-Methylquinolin-4-ylamino)-phenyl]-ethanone (**2j**)*. Conditions were as employed for **2b**. 3-Aminoacetophenone **j** (347 mg, 2.6 mmol), **2** (229 mg, 1.3 mmol), reaction time 18 h, gave **2j** as a solid. (348 mg, 82%). m.p., 210 °C (dec); IR (Solid): ν_{max} cm^{-1}, 1686, 1636, 1585, 1556, 1447, 1360, 1294, 1260, 1204; ^1H NMR (250 MHz, d^6-DMSO): δ 2.67 (3H, s, CH_3), 2.68 (3H, s, CH_3), 6.81 (1H, s, Ar-H), 7.72–7.84 (3H, m, Ar-H), 8.00–8.13 (4H, m, Ar-H), 8.81 (1H, d, $J = 8.2$, Ar-H), 10.94 (1H, s, NH); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 19.8, 26.9, 106.8, 155.6, 116.3, 119.8, 123.5, 124.9, 126.7, 126.8, 125.9, 130.1, 130.4, 130.9, 130.9, 134.1, 154.1, 155.4; ESI-MS *m/z:* 277 $[M + H]^+$; found 277.1344 ($C_{18}H_{17}N_2O_2$ $[M + H]^+$, requires 277.1341).

*6.1.2.10. 4-(2-Methylquinolin-4-ylamino)-benzoic acid (**2k**)*. Conditions were as employed for **1a**. 4-Aminobenzoic acid **k** (152 mg, 1.1 mmol), **5** (208 mg, 1.2 mmol), reaction time 5 h, gave **2k** after basic extraction as a solid. (107 mg, 37%). m.p., > 300 °C; IR (Solid): ν_{max} cm^{-1}, 1692, 1639, 1588, 1524, 1439, 1369, 1277, 1220, 1169; ^1H NMR (250 MHz, d^6-DMSO): δ 2.70 (3H , s, CH_3), 7.01 (1H, s, Ar-H), 7.67 (2H, d, $J = 8.6$, Ar-H), 7.84 (1H, t, $J = 6.7$, Ar-H), 8.02 (1H, t, $J = 7.9$, Ar-H), 8.11–8.17 (3H, m, Ar-H), 8.85 (1H, d, $J = 8.5$, Ar-H), 11.0 (1H, bs, NH); ^{12}C NMR (62.5 MHz, d^6-DMSO): δ 19.1, 101.0, 116.6, 119.8, 123.7, 124.5, 126.8, 128.7, 131.0, 133.8, 138.8, 141.8, 153.6, 155.1, 166.7; ESI-MS *m/z:* 279 $[M + H]^+$; found 279.1140 ($C_{17}H_{15}N_2O_2$ $[M + H]^+$, requires 279.1134).

*6.1.2.11. (2-Methylquinolin-4-yl)-(3-phenoxyphenyl)-amine (**2m**)*. Conditions were as employed for **2b**. 3-Phenoxyaniline **m** (367.6 mg, 1.98 mmol), **5** (0.20 ml, 1.00 mmol), reaction time 18 h, gave product **2m** (222.3 mg, 61%) as a solid. m.p., 229 °C; IR (Solid): ν_{max} cm^{-1}, 3013.9, 2672.0, 1640.8, 1605.9, 1582.4, 1554.3, 1533.6, 1482.7, 1440.1, 1367.4, 1293.7, 1253.2, 1208.6, 1153.0, 1142.6, 1099.2, 1068.4, 1020.7; ^1H NMR (250 MHz, d^6-DMSO): δ 2.69 (3H, s, CH_3), 6.87 (1H, s, Ar-H), 6.87–7.33 (7H, m, Ar-H), 7.60 (2H, t, $J = 7.8$, Ar-H), 7.77 (1H, t, $J = 8.1$, Ar-H), 8.02 (1H, t, $J = 7.6$, Ar-H), 8.16 (1H, d, $J = 8.2$, Ar-H), 8.81 (1H, d, $J = 8.5$, Ar-H), 10.90 (1H, s, NH); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 19.8, 100.4, 106.9, 114.8, 116.3, 116.9, 119.2, 119.7, 123.6, 124.1, 126.5, 130.2, 131.2, 133.6, 138.4, 138.9, 154.0, 154.7, 156.0, 156.0; ESI-MS *m/z:* 327 $[M + H]^+$; found 327.1490 ($C_{22}H_{19}N_2O$ $[M + H]^+$, requires 327.1497).

*6.1.2.12. 3-(2-Methylquinolin-4-ylamino)-benzonitrile (**2n**)*. Conditions were as employed for **2b**. 3-Aminobenzonitrile **n** (243.0 mg, 2.06 mmol), **5** (0.20 ml, 1.00 mmol), reaction time 18 h, gave product **2n** (135.7 mg, 46%) as a solid. m.p., 298 °C; IR (Solid): ν_{max} cm^{-1}, 2849.2, 2230.4, 1639.1, 1583.4, 1557.2, 1486.1, 1444.2, 1393.1, 1368.7, 1280.6, 1246.0, 1208.0 and 1143.3; ^1H NMR (250 MHz, d^6-DMSO): δ 1.96 (3H, s, CH_3), 6.07 (1H, s, Ar-H), 6.79 (7H, m, Ar-H), 7.24

(1H, d, $J = 8.5$); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 19.8, 100.7, 112.7, 116.4, 118.2, 118.9, 121.7, 123.7, 126.7, 128.6, 130.3, 130.4, 131.2, 133.8, 138.4, 153.9, 155.2; ESI-MS *m/z:* 260 $[M + H]^+$; found 260.1194 ($C_{17}H_{14}N_3$ $[M + H]^+$, requires 260.1188).

*6.1.2.13. 2-Methyl-4-phenoxyquinoline (**2y**).* Procedure similar to that employed for **1l**. 4-Amino-6-methoxypyrimidine **l** (69 mg, 0.5 mmol), **5** (101 mg, 0.6 mmol) and phenol (1 g). **2y** was isolated after column chromatography (ethyl acetate/hexane 1:4) as a solid. (77 mg, 55%). m.p., 77 °C; IR (Solid): ν_{max} cm^{-1}, 3309, 3244, 2926, 1619, 1590, 1509, 1486, 1415, 1378, 1344, 1232, 1209, 1178 and 1069; ^{1}H NMR (250 MHz, $CDCl_3$): δ 2.6 (3H, s, CH_3), 6.4 (1H, s, Ar-H), 7.1–7.2 (2H, m, Ar-H), 7.3 (1H, d, $J = 6.9$, Ar-H), 7.4–7.5 (3H, m, Ar-H), 7.7 (1H, t, $J = 6.3$, Ar-H), 8.0 (1H, d, $J = 7.5$, Ar-H), 8.3 (1H, d, $J = 7.5$, Ar-H); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 104.8, 121.1, 121.9, 125.4, 125.6, 130.3; ESI-MS *m/z:* 236 $[M + H]^+$; found 236.106 ($C_{16}H_{13}ON.HCl$ $[M + H]^+$, requires 236.107).

6.1.3. 2-Phenylquinazolines

*6.1.3.1. Phenyl-(2-phenylquinazolin-4-yl)-amine (**3a**).* Procedure **D.** Aniline 7**a** (97 mg, 1.0 mmol), **6** (121 mg, 0.5 mmol), reaction time 4 h, gave **3a** as a solid. (134 mg, 80%). m.p., 239–242 °C; IR (Solid): ν_{max} cm^{-1}, 3046, 2332, 1608, 1546; ^{1}H NMR (250 MHz, d^6-DMSO): δ 7.40 (1H, t, $J = 7.3$, Ar-H), 7.59 (2H, t, $J = 7.9$, Ar-H), 7.61–7.80 (2H, m, Ar-H), 7.69 (2H, d, $J = 7.6$, Ar-H), 7.84 (1H, s, NH), 7.90 (2H, d, $J = 7.9$, Ar-H), 8.13 (1H, t, $J = 7.6$, Ar-H), 8.42 (1H, d, $J = 7.0$, Ar-H), 8.43 (2H, d, $J = 7.0$, Ar-H), 9.00 (1H, d, $J = 7.9$, Ar-H), 11.71 (1H, s, NH); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 106.7, 112.8, 124.5, 124.6, 126.4, 128.1, 128.2, 128.7, 129.0, 129.2, 133.2, 135.8, 137.0, 157.3, 159.0; ESI-MS *m/z:* 298 $[M + H]^+$; found 298.1345 ($C_{20}H_{16}N_3$ $[M + H]^+$, requires 298.1344).

*6.1.3.2. (3-Ethylphenyl)-(2-phenylquinazolin-4-yl)-amine (**3b**).* Conditions were as employed for **3a**. 3-Ethylaniline **b** (250.6 mg, 2.06 mmol), **6** (232.5 mg, 0.96 mmol), reaction time 5 h, gave product **3b** (321.6 mg, 92%) as a solid. m.p., 158 °C; IR (Solid): ν_{max} cm^{-1}, 2827.8, 2341.5, 2228.8, 2030.3, 1628.2, 1609.5, 1550.4, 1503.2, 1487.4, 1457.3, 1414.9, 1361.4, 1329.4, 1147.0; ^{1}H NMR (250 MHz, d^6-DMSO): δ 1.28 (3H, t, $J = 7.5$, CH_3), 2.71 (2H, q, $J = 7.5$, CH_2), 7.21 (1H, d $J = 7.6$, Ar-H), 7.45 (1H, t, $J = 7.8$, Ar-H), 7.59–7.85 (6H, m, Ar-H), 8.10 (1H, t, $J = 7.9$, Ar-H), 8.46–8.55 (3H, m, Ar-H), 9.07 (1H, d, $J = 8.2$, Ar-H), 11.87 (1H, s, NH); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 15.6, 28.2, 112.6, 120.2, 121.2, 123.9, 124.7, 126.0, 128.0, 128.5, 128.8, 129.3, 131.2, 133.4, 135.5, 136.7, 140.2, 144.2, 156.8, 158.8; ESI-MS *m/z:* 326 $[M + H]^+$; found 326.1667 ($C_{22}H_{20}N_3$ $[M + H]^+$, requires 326.1657).

*6.1.3.3. (3-Methylsulphanylphenyl)-(2-phenylquinazolin-4-yl)-amine (**3c**).* Conditions were as employed for **3a**. 3-(Methylmercapto) aniline **c** (0.13 ml, 1.0 mmol), **6** (121 mg, 0.5 mmol, reaction time 22 h, gave **3c** as a solid. (148 mg, 78%). m.p., 171–175 °C; IR (Solid) ν_{max} cm^{-1}, 3047, 2628, 1598, 1557, 1356; ^{1}H NMR (250 MHz, d^6-DMSO): δ 2.53 (3H, s, SCH_3), 7.24 (1H, d, $J = 7.9$, Ar-H), 7.48 (1H, t, $J = 7.9$, Ar-H), 7.60–7.77 (5H, m, Ar-H), 7.83 (1H, t, $J = 7.6$, Ar-H), 7.87 (1H, s, Ar-H), 8.11 (1H, t, $J = 7.9$, Ar-H), 8.42 (1H, s, Ar-H), 8.44 (2H, d, $J = 7.3$, Ar-H), 9.03 (1H, d, $J = 8.2$, Ar-H), 11.76 (1H, s, NH); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 14.7, 106.8, 112.8, 120.8, 121.5, 123.6, 124.6, 126.0, 128.1, 129.0, 129.2, 129.2, 131.0, 133.3, 135.9, 137.6, 138.7, 157.2, 159.0; ESI-MS *m/z:* 344 $[M + H]^+$; found 344.1210 ($C_{21}H_{18}N_3S$ $[M + H]^+$, requires 344.1221).

*6.1.3.4. (3-Methoxyphenyl)-(2-phenylquinazolin-4-yl)-amine (**3d**).* Conditions were as employed for **3a**. *m*-Anisidine **d** (496.1 mg, 2.01 mmol), **6** (269.9 mg, 1.12 mmol), reaction time 18 h, gave product **3d** (402.9 mg, 99%) as a solid. m.p., 232 °C; IR (Solid) ν_{max} cm^{-1}, 2889.9, 2835.0, 1632.8, 1599.7, 1555.9, 1504.2, 1456.6, 1358.4, 1331.4, 1326.9, 1296.9, 1243.6, 1168.2, 1026.2; ^{1}H NMR (250 MHz, d^6-DMSO): δ 3.86 (3H, s, OCH_3), 7.13 (2H, d, $J = 9.2$, Ar-H), 7.64–7.87 (6H, m, Ar-H), 8.11 (1H, t, $J = 7.6$, Ar-H), 8.40 (3H, d, $J = 7.6$, Ar-H), 8.97 (1H, d, $J = 8.2$, Ar-H), 11.69 (1H, s, NH); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 55.4, 109.9, 112.2, 112.9, 116.5, 124.6, 129.0, 129.2, 129.6, 135.8, 138.3, 157.4, 157.6, 159.0, 159.5; ESI-MS *m/z:* 328 $[M + H]^+$; found 328.1435 ($C_{21}H_{18}N_3O$ $[M + H]^+$, requires 328.1450).

*6.1.3.5. (4-Methoxyphenyl)-(2-phenylquinazolin-4-yl)-amine (**3e**).* Conditions were as employed for **3a**. *p*-Anisidine **e** (496.1 mg, 2.01 mmol), **6** (234.3 mg, 0.97 mmol), reaction time 18 h, gave product **3e** (303.1 mg, 86%) as a solid. m.p., 248 °C; IR (Solid) ν_{max} cm^{-1}, 2836.5, 2656.5, 1627.6, 1627.6, 1610.2, 1559.8, 1495.9, 1457.1, 1435.9, 1421.4, 1370.6, 1358.7, 1329.6, 1294.4, 1264.1, 1186.7, 1152.5, 1032.1; ^{1}H NMR (250 MHz, d^6-DMSO): δ 3.86 (3H, s, OCH_3), 6.96 (1H, d t, $J = 6.7$, $J = 2.5$, Ar-H), 7.44–7.49 (2H, m, Ar-H), 7.59–7.83 (4H, m, Ar-H), 7.90 (1H, t, $J = 7.7$, Ar-H), 8.13 (1H, t, $J = 7.6$, Ar-H), 8.38–8.46 (3H, m, Ar-H), 8.97 (1H, d, $J = 8.24$, Ar-H), 11.55 (1H, s, NH); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 55.4, 109.9, 112.2, 112.9, 124.6, 129.0, 129.2, 129.6, 135.8, 138.4, 157.32, 159.0, 159.5; ESI-MS *m/z:* 328 $[M + H]^+$; found 328.1456 ($C_{22}H_{18}N_3O$ $[M + H]^+$, requires 328.1450).

*6.1.3.6. [4-(2-Phenylquinazolin-4-ylamino)-phenyl]-methanol (**3f**).* Conditions were as employed for **3a**. 3-Aminobenzylalcohol **f** (246.2 mg, 2.00 mmol), **6** (271.9 mg, 1.16 mmol), reaction time 18 h, gave product **3f** (396.6 mg, 96%) as a solid. m.p., 240 °C; IR (Solid): ν_{max} cm^{-1}, 2828.0, 2230.3, 1971.3, 1590.8, 1551.7, 1502.5, 1458.0, 1419.7, 1367.9, 1328.1, 1164.8, 1021.4; ^{1}H NMR (250 MHz, d^6-DMSO): δ 4.65 (2H, s, CH_2OH), 7.27 (1H, d, $J = 4.6$, Ar-H), 7.52 (1H, t, $J = 7.6$, Ar-H), 7.76–7.79 (4H, m, Ar-H), 7.86 (1H, t, $J = 7.3$, Ar-H), 7.97 (1H, s, Ar-H), 8.12 (1H, t, $J = 7.6$, Ar-H), 8.24 (1H, d, $J = 7.9$, Ar-H), 8.43 (2H, d, $J = 7.6$, Ar-H), 8.86 (1H, d, $J = 9.5$, Ar-H); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 55.3,

111.3, 114.7, 115.0, 115.7, 117.2, 120.5, 120.5, 120.7, 120.9, 122.9, 125.5, 128.1, 128.5; ESI-MS *m/z:* 328 $[M + H]^+$; found 328.1458 ($C_{21}H_{18}N_3O$ $[M + H]^+$, requires 328.1450).

*6.1.3.7. 4-(2-Phenylquinazolin-4-ylamino)-phenol (**3g**)*. Conditions were as employed for **3a**. 4-Aminophenol **g** (220.5 mg, 2.02 mmol), **6** (252.5 mg, 1.05 mmol), reaction time 18 h, gave product **3g** (347.9 mg, 96%) as a solid. m.p., 241 °C decomposed; IR(Solid) ν_{max} cm^{-1}, 2911.4, 2591.1, 1627.3, 1601.1, 1557.0, 1501.7, 1450.1, 1421.9, 1367.5, 1267.7, 1218.4, 1187.4, 1170.0, 1107.1, 1030.2; ^1H NMR (250 MHz, d^6-DMSO): δ 6.89 (1H, d, $J = 8.9$, Ar-H), 6.96 (2H, d, $J = 8.9$, Ar-H), 7.23 (1H, d, $J = 8.9$, Ar-H), 7.62–7.78 (4H, m, Ar-H), 7.83 (1H, t, $J = 7.6$, Ar-H), 8.10 (1H, t, $J = 7.8$, Ar-H), 8.38 (3H, m, Ar-H), 8.89 (1H, d, $J = 7.9$, Ar-H), 11.46 (1H, s, NH); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 112.6, 115.2, 116.0, 122.3, 124.3, 126.0, 127.9, 129.0, 129.1, 131.5, 133.8, 134.9, 155.5, 157.2, 159.2; ESI-MS *m/z:* 314 $[M + H]^+$; found 314.1293 ($C_{20}H_{16}N_3O$ $[M + H]^+$, requires 314.1293).

*6.1.3.8. (3-Fluoro-4-methoxyphenyl)-(2-phenylquinazolin-4-yl)-amine (**3h**)*. Conditions were as employed for **3a**. 3-Fluoro-4-methoxy aniline **h** (85 mg, 0.6 mmol) was reacted with **6** (72 mg, 0.3 mmol), reaction time 19 h, gave **3h** as a solid. (114 mg, 99%). m.p., 219–222 °C; IR (Solid): ν_{max} cm^{-1}, 2781, 1632, 1502, 1269, 1026; ^1H NMR (250 MHz, d^6-DMSO): δ 4.37 (3H, s, OCH_3), 7.60–7.85 (2H, m, Ar-H), 8.01–8.22 (3H, m, Ar-H), 8.22–8.35 (2H, m, Ar-H), 8.54 (1H, t, $J = 7.6$, Ar-H), 8.78 (1H, s, Ar-H), 8.83 (2H, d, $J = 7.0$, Ar-H), 9.40 (1H, d, $J = 8.5$, Ar-H), 12.12 (1H, s, NH); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 56.2, 102.0, 112.7, 113.5, 118.0, 120.6, 124.5, 128.1, 129.0, 129.1, 129.8, 131.8, 133.2, 135.8, 145.4, 152.5, 157.3, 158.8; ESI-MS *m/z:* 346 $[M + H]^+$; found 346.1347 ($C_{21}H_{17}FN_3O$ $[M + H]^+$, requires 346.1356).

*6.1.3.9. (3-Fluorophenyl)-(2-phenylquinazolin-4-yl)-amine (**3i**)*. Conditions were as employed for **3a**. 3-Fluroaniline **i** (219.5 mg, 1.97 mmol), **6** (232.1 mg, 0.96 mmol), reaction time 18 h, gave product **3i** (299.0 mg, 89%) as a solid. m.p., 278 °C; IR (Solid): ν_{max} cm^{-1}, 3049.4, 2681.7, 1626.3, 1598.3, 1574.0, 1545.9, 1485.0, 1456.0, 1419.6, 1360.7, 1326.7, 1256.3, 1139.4; ^1H NMR (250 MHz, d^6-DMSO): δ 7.2 (1H, t, $J = 7.3$, Ar-H), 7.5–8.00 (7H, m, Ar-H), 8.1 (1H, t, $J = 7.8$, Ar-H), 8.2 (1H, d, $J = 7.9$, Ar-H), 8.4 (2H, d, $J = 7.5$, Ar-H), 8.9 (1H, d, $J = 7.6$, Ar-H); ^1H NMR (62.5 MHz, d^6-DMSO): δ 113.0, 124.4, 129.0, 130.4, 130.2, 134.0, 135.7, 157.6, 159.0; ESI-MS *m/z:* 316 $[M + H]^+$; found 316.1251 ($C_{20}H_{15}N_3F$ $[M + H]^+$, requires 316.1250).

*6.1.3.10. 1-[3-(2-Phenylquinazolin-4-ylamino)-phenyl]-ethanone (**3j**)*. Conditions were as employed for **3a**. 3-Aminoacetophenone **j** (136 mg, 1.0 mmol), **6** (122 mg, 0.5 mmol), reaction time 6 h, gave **3j** as a solid. (170 mg, 89%). m.p., 199–204 °C; IR (Solid) ν_{max} cm^{-1}, 2500, 1673, 1636, 1567; ^1H NMR (250 MHz, d^6-DMSO): δ 2.68 (3H, s, OCH_3), 7.62–7.79 (4H, m, Ar-H), 7.87 (1H, t, $J = 7.6$, Ar-H), 7.98 (1H, d, $J = 7.9$, Ar-H), 8.14 (1H, t, $J = 7.6$, Ar-H), 8.19 (1H, d, $J = 9.2$, Ar-H), 8.44 (1H, d, $J = 9.2$, Ar-H), 8.48 (2H, d, $J = 7.0$, Ar-H), 8.62 (1H, s, Ar-H), 9.05 (1H, d, $J = 8.2$, Ar-H), 11.86 (1H, s, NH); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 106.8, 112.9, 123.6, 124.6, 126.0, 128.1, 128.7, 129.0, 129.1, 129.3, 130.0, 131.1, 133.3, 135.9, 137.2, 137.6, 157.3, 159.0, 197.5; ESI-MS *m/z:* 340 $[M + H]^+$; found 340.1437 ($C_{22}H_{18}N_3O$ $[M + H]^+$, requires 340.1450).

*6.1.3.11. 4-(2-Phenylquinazolin-4-ylamino)-benzoic acid (**3k**)*. Conditions were as employed for **3a**. 4-Aminobenzoic acid **k** (210 mg, 1.0 mmol), **6** (122 mg, 0.5 mmol), reaction time 26 h, gave **3k** as a solid. (188 mg, 98%). m.p., 281–284 °C; IR (Solid): ν_{max} cm^{-1}, 2904, 1709, 1598, 1542; ^1H NMR (250 MHz, d^6-DMSO): δ 7.64–7.80 (4H, m, Ar-H), 7.88 (1H, t, $J = 7.6$, Ar-H), 8.05–8.19 (5H, m, Ar-H), 8.46 (2H, d, $J = 8.5$, Ar-H), 8.45 (1H, s, OH), 9.06 (1H, d, $J = 8.2$, Ar-H), 11.87 (1H, s, NH); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 107.9, 113.0, 123.9, 124.7, 127.9, 128.2, 129.1, 129.3, 130.0, 131.1, 132.6, 133.3, 136.0, 141.2, 157.3, 159.1, 166.8; ESI-MS *m/z:* 342 $[M + H]^+$; found 342.1243 ($C_{21}H_{16}N_3O_2$ $[M + H]^+$, requires 342.1243).

*6.1.3.12. (6-Methoxypyrimidin-4-yl)-(2-phenylquinazolin-4-yl)-amine (**3l**)*. Procedure **C**. 6-Methoxypyrimidin-4-ylamine **7l** (300.0 mg, 2.40 mmol), **6** (112.0 mg, 0.47 mmol), reaction time 70 h. Purification by flash column chromatography (1:1 EtOAc/hexane, $R_f = 0.43$) gave **3l** as a yellow crystalline solid (5 mg, 3% yield). IR (Solid): ν_{max} cm^{-1} 3423, 2952, 2927, 1699, 1597, 1555, 1509, 1471, 1441, 1384, 1350, 1327, 1297, 1195, 1160; ^1H NMR (250 MHz, $CDCl_3$): δ 4.0 (3H, s, OMe), 7.45–7.57 (4H, m, Ar-H), 7.79 (1H, t, $J = 7.0$, Ar-H), 7.90–8.02 (2H, m, Ar-H), 8.23 (1H, s, Ar-H), 8.32 (1H, s, NH), 8.14 (1H, s, Ar-H), 8.48 (2H, d, $J = 5.6$, Ar-H); ESI-MS *m/z:* 330 $[M + H]^+$; found 330.1354 ($C_{19}H_{16}N_5O$ $[M + H]^+$ requires 330.1355).

*6.1.3.13. (3-Phenoxyphenyl)-(2-phenylquinazolin-4-yl)-amine (**3m**)*. Conditions were as employed for **3a**. 3-Phenoxyaniline **m** (367.6 mg, 1.98 mmol), **6** (226.1 mg, 0.94 mmol), reaction time 18 h, gave product **3m** (281.5 mg, 70%) as a solid. m.p., 282 °C decomposed; IR (Solid): ν_{max} cm^{-1}, 2909.6, 2230.0, 1627.6, 1600.2, 1553.9, 1503.4, 1488.5, 1455.8, 1419.7, 1365.2, 1331.3, 1267.6, 1217.1, 1167.1; ^1H NMR (250 MHz, d^6-DMSO): δ 6.25–6.37 (4H, m, Ar-H), 6.51–6.59 (2H, m, Ar-H), 6.72–6.85 (5H, m, Ar-H), 6.95 (1H, t, $J = 7.5$, Ar-H), 7.07 (1H, td, $J = 8.1$, $J = 1.4$, Ar-H), 7.29 (1H, d, $J = 4.0$, Ar-H), 7.29 (1H, t, $J = 8.3$, Ar-H), 7.41 (1H, s, Ar-H), 7.43 (1H, d, $J = 7.3$, Ar-H), 7.84 (1H, d, $J = 7.9$, Ar-H); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 104.6, 106.5, 109.1, 110.6, 110.7, 111.5, 115.4, 115.6, 120.5, 120.6, 120.9, 121.6, 121.8, 125.4, 128.1, 129.8; ESI-MS *m/z:* 390 $[M + H]^+$; found 390.1595 ($C_{26}H_{20}N_3O$ $[M + H]^+$, requires 390.1606).

*6.1.3.14. 3-(2-Phenylquinazolin-4-ylamino)-benzonitrile (**3n**)*. Conditions were as employed for **3a**. 3-Aminobenzonitrile **n** (243.0 mg, 2.55 mmol), **6** (253.3 mg, 1.05 mmol), reaction time 18 h, gave product **3n** (300.5 mg, 80%) as a solid. m.p.,

260 °C; IR (Solid): ν_{max} cm^{-1}, 2827.7, 2231.6, 1630.5, 1592.5, 1556.7, 1505.4, 1458.3, 1443.8, 1420.0, 1374.0, 1330.0, 1164.7; ^1H NMR (250 MHz, d^6-DMSO): δ 7.63–7.93 (6H, m, Ar-H), 8.14 (1H, t, $J = 7.6$, Ar-H), 9.02 (1H, d, $J = 7.6$, Ar), 8.36–8.43 (4H, m, Ar-H), 9.02 (1H, d, $J = 8.2$, Ar-H), 11.90 (1H, s, NH); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 111.5, 112.9, 118.5, 124.6, 127.5, 128.1, 129.0, 129.1, 130.1, 133.1, 135.9, 138.2, 157.5, 159.1, 209.4; ESI-MS *m/z:* 323 $[M + H]^+$; found 323.1283 ($C_{21}H_{15}N_4$ $[M + H]^+$, requires 323.1297).

6.1.3.15. N-(2-Phenylquinazolin-4-yl)-benzene-1,4-diamine ***(3o)***. Conditions were as employed for **3a**. 1,3-Phenylenediamine **o** (217.4 mg, 2.00 mmol), **3** (222.3 mg, 1.08 mmol), reaction time 18 h, gave product **3o**, (51.8 mg, 17%) as a solid. m. p. 201 °C, IR (Solid): ν_{max} cm^{-1}, 2909.6, 2230.0, 1627.6, 1600.2, 1553.9, 1503.4, 1488.5, 1455.8, 1419.7, 1365.2, 1331.3, 1267.6, 1217.1, 1167.1; ^1H NMR (250 MHz, d^6-DMSO): δ 6.62 (1H, dt, $J = 7.3$, $J = 2.0$, Ar-H), 7.18 (1H, dd, $J = 3.7$, $J = 1.8$, Ar-H), 7.47–7.53 (6H, m, Ar-H), 7.78 (1H, td, $J = 7.6$, $J = 1.3$, Ar-H), 7.86 (1H, d, $J = 8.2$, Ar-H), 8.0 (1H, d, $J = 8.5$, Ar-H), 8.52–8.57 (2H, m, Ar-H); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 107.6, 108.2, 111.1, 111.5, 114.1, 120.4, 126.2, 128.6, 128.7, 129.4, 130.0, 130.4, 133.0, 139.8, 147.4, 157.5; ESI-MS *m/z:* 313 $[M + H]^+$; found 313.1462 ($C_{20}H_{17}N_4$ $[M + H]^+$, requires 313.1463).

6.1.3.16. (2-Phenylquinazolin-4-yl)-pyridin-3-ylmethylamine ***(3p)***. Conditions were as employed for **3l** although heated at 70 °C. 3-Picolylamine **p** (265.0 mg, 2.45 mmol), 6 (121.0 mg, 0.50 mmol), reaction time 3 h, gave **3p** as a yellow crystalline solid (137 mg, 88%). m.p., 216–217 °C; IR (Solid): ν_{max} cm^{-1} 3253, 3033, 2932, 1563, 1532, 1484, 1424, 1363, 1322, 1180, 1031; ^1H NMR (250 MHz, DMSO): δ 4.92 (2H, d, $J = 5.5$ Hz, N-CH_2), 7.34 (1H, dd, $J = 7.6$ Hz, J 4.6 Hz, Ar-H), 7.47 (2H, t, $J = 3.7$ Hz, Ar-H), 7.48–7.60 (2H, m, Ar-H), 7.78 (2H, d, $J = 4.0$ Hz, Ar-H), 7.85 (1H, d, $J = 7.9$ Hz, Ar-H), 8.27 (1H, d, $J = 8.2$ Hz, Ar-H), 8.44 (2H, d, $J = 3.7$ Hz, Ar-H), 8.41–8.47 (1H, m, Ar-H), 8.72 (1H, d, $J = 1.8$ Hz, N-CH) 9.02 (1H, t, $J = 5.8$ Hz, NH); ^{13}C NMR (62.5 MHz, DMSO): δ 41.6, 113.8, 122.7, 123.5, 125.5, 127.8, 127.9, 128.3, 130.2, 132.9, 135.2, 138.4, 148.1, 149.0, 149.8, 159.1, 159.5; ESI-MS *m/z:* 313 $[M + H]^+$; found 313.1467 ($C_{20}H_{17}N_4$ $[M + H]^+$, requires 313.1453).

6.1.3.17. Furan-2-ylmethyl-(2-phenylquinazolin-4-yl)-amine ***(3q)***. Conditions were as employed for **3a** although heated at 98 °C. Furfurylamine **q** (241.5 mg, 2.49 mmol), **6** (122.0 mg, 0.51 mmol), reaction time 3 h, gave **3q** as a yellow crystalline solid (126 mg, 84%). m.p., 119–121 °C; IR (Solid): ν_{max} cm^{-1} 3360, 3122, 3047, 2908, 1619, 1563, 1530, 1423, 1376, 1342, 1205, 1149, 1009, 928; ^1H NMR (250 MHz, $CDCl_3$): δ 4.99 (2H, d, $J = 5.2$ Hz, CH_2), 6.03 (1H, t, $J = 4.3$ Hz, NH), 6.33–6.42 (2H, m, Ar-H), 7.39 (2H, t, $J = 8.2$ Hz, Ar-H), 7.45–7.57 (3H, m, Ar-H), 7.68 (1H, d, $J = 9.2$ Hz, Ar-H), 7.71 (1H, td, $J = 7.0$ Hz, J 1.5 Hz, Ar-H), 7.93 (1H, d, $J = 8.2$ Hz, Ar-H), 8.55–8.64 (2H, d, $J = 8.2$ Hz, Ar-H); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 38.2, 108.0, 110.6, 113.6, 120.6, 125.5, 128.3, 128.4, 128.9, 130.2, 132.7, 138.9, 142.3, 150.6; ESI-MS *m/z:* 302 $[M + H]^+$; found 302.1283 ($C_{19}H_{16}N_3O$ $[M + H]^+$, requires 302.1293).

6.1.3.18. Cyclohexyl-(2-phenylquinazolin-4-yl)-amine ***(3r)***. Conditions were as employed for **3l** although heated at 98 °C. Cyclohexylamine **r** (247.9 mg, 2.45 mmol), **6** (120.4 mg, 0.50 mmol), reaction time 2.5 h, gave **3r** as a yellow crystalline solid (130 mg, 86%). m.p., 154–155 °C; IR (Solid): ν_{max} cm^{-1} 3325, 3060, 2926, 2846, 1619, 1560, 1517, 1420, 1367; ^1H NMR (250 MHz, $CDCl_3$): δ 1.10–1.85 (10H, m, C-CH_2-C), 4.34 (1H, dtt, $J = 7.3$, $J = 7.0$, $J = 4.0$, N-CH), 5.47 (1H, d, $J = 7.0$, NH), 7.31 (1H, t, $J = 7.0$, Ar-H), 7.36–7.47 (3H, m, Ar-H), 7.54–7.68 (2H, m, Ar-H), 7.88 (1H, d, $J = 7.9$, Ar-H), 8.47 (2H, d, $J = 7.9$, Ar-H); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 25.1, 25.8, 33.0, 49.8, 107.5, 113.7, 120.4, 125.2, 128.3, 128.4, 129.0, 130.0, 132.4, 139.1, 150.6, 158.8; ESI-MS *m/z:* 304 $[M + H]^+$; found 304.813 ($C_{20}H_{22}N_3$ $[M + H]^+$, requires 304.1814).

6.1.3.19. 2-Phenyl-4-piperidin-1-ylquinazoline ***(3s)***. Conditions were as employed for **3l**. Piperidine **s** (2.5 mmol), **6** (120 mg, 0.5 mmol), reaction time 0.3 h, gave **3s** as a yellow crystalline solid (123 mg, 84%). m.p., 106 °C; IR (Solid): ν_{max} cm^{-1} 3062, 2930, 2817, 1611, 1563, 1502, 1433, 1353, 1109; ^1H NMR (250 MHz, $CDCl_3$): δ 1.75 (6H, s, C-CH_2-C), 3.74 (4H, s, N-CH_2-C), 7.32 (1H, t, $J = 7.0$ Hz, Ar-H), 7.36–7.48 (3H, m, Ar-H), 7.63 (1H, t, $J = 7.7$ Hz, Ar-H), 7.81 (1H, d, $J = 8.2$ Hz, Ar-H), 7.92 (1H, d, $J = 8.2$ Hz, Ar-H), 8.49 (2H, d, $J = 7.9$ Hz, Ar-H); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 24.9, 26.1, 51.0, 124.6, 125.1, 128.3, 128.4, 128.6, 130.1, 132.3; ESI-MS *m/z:* 290 $[M + H]^+$; found 290.1654 ($C_{19}H_{20}N_3$ $[M + H]^+$, requires 290.1657).

6.1.3.20. Allyl-(2-phenylquinazolin-4-yl)-amine ***(3t)***. Conditions were as employed for **3l** although heated at 50 °C. Allylamine **t** (144.3 mg, 2.53 mmol), **6** (122.4 mg, 0.51 mmol), reaction time 19 h gave **3t** as a yellow crystalline solid (120 mg, 90%). m.p., 128 °C; IR (Solid): ν_{max} cm^{-1} 3303, 3060, 2929, 1618, 1563, 1529, 1436, 1412, 1374, 1322; ^1H NMR (250 MHz, $CDCl_3$): δ 4.37 (2H, d, $J = 5.5$, CH_2–C=C), 5.16 (1H, dq, $J_{trans} = 9.9$, $J = 1.2$, C=CH_2), 5.28 (1H, dq, $J_{cis} = 17.4$, $J = 1.5$, C=CH_2), 5.72 (1H, s, NH), 6.02 (1H, dtt, , $J = 5.8$, $J_{cis} = 17.1$, $J_{tans} = 10.4$, *H*C=CH_2), 7.33 (1H, t, $J = 7.0$, Ar-H), 7.35–7.46 (3H, m, Ar-H), 7.65 (2H, t, $J = 5.5$, Ar-H), 7.85 (1H, d, $J = 8.2$, Ar-H), 8.49 (2H, m, Ar-H); ^{13}C NMR (62.5 MHz, $CDCl_3$) 43.8, 117.0, 120.4, 125.5, 128.3, 128.4, 128.9, 130.1, 132.6, 134.5; ESI-MS *m/z:* 262 $[M + H]^+$; found 262.1343 ($C_{17}H_{16}N_3$ $[M + H]^+$, requires 262.1344).

6.1.3.21. N,N-dimethyl-N′-(2-phenylquinazolin-4-yl)-ethane-1,2-diamine ***(3u)***. Conditions were as employed for **3l** although heated at 98 °C. *N′*,*N′*-Dimethylethane-1,2-diamine **7u** (120.0 mg, 0.50 mmol), 3 (192.7 mg, 2.19 mmol), reaction

time 10 min, gave **3u** as a viscous orange oil (121 mg, 83%). IR (Solid): ν_{max} cm^{-1} 2942, 2823, 1618, 1572, 1530, 1454, 1430, 1413, 1363, 1274, 1219, 1202, 1070; ^1H NMR (250 MHz, $CDCl_3$): δ 2.25 (6H, s, N-(CH_3)$_2$), 2.59 (2H, t, J = 5.8 Hz, CH_2-N-Me$_2$), 3.75 (2H, q, J = 5.2 Hz, CH_2-NH), 6.50 (1H, br-t, NH), 7.32 (1H, t, J = 7.0 Hz, Ar), 7.35–7.45 (2H, m, Ar-H), 7.62 (1H, t, J = 8.2 Hz, Ar-H), 7.69 (1H, d, J = 7.9 Hz, Ar-H), 7.82 (1H, d, J = 8.2 Hz, Ar-H), 8.5 (2H, dd, J = 7.0 Hz, J = 2.4 Hz, Ar-H); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 38.3, 45.3, 57.6, 90.3, 107.5, 113.9, 121.0, 125.3, 128.2, 128.4, 128.7, 130.0, 132.4, 139.1, 150.4, 160.6; ESI-MS *m/z:* 293 $[M + H]^+$; found 293.1776 ($C_{18}H_{21}N_4$ $[M + H]^+$, requires 293.1772).

*6.1.3.22. N_1-(2-Phenylquinazolin-4-yl)-butane-1,4-diamine (**3v**)*. Conditions for synthesis were as applied to compound **3u**. Butane-1,4-diamine **v** (219.3 mg, 2.49 mmol), **6** (120.0 mg, 0.50 mmol), reaction time 0.5 h, gave **3v** as a yellow crystalline solid (89 mg, 61%). m.p., 90–95 °C; IR (Solid): ν_{max} cm^{-1} 2932, 2855, 1592, 1572, 1531, 1489, 1454, 1436, 1372, 1322, 1260, 1166; ^1H NMR (250 MHz, $CDCl_3$): δ 1.46–1.60 (4H, m, CH_2), 1.72 (2H, qu, J = 6.7, CH_2), 2.70 (2H, t, J = 6.4, CH_2), 3.67 (2H, td, J = 6.4, J = 5.2, HN-CH_2), 6.8 (1H, s, NH), 7.26 (1H, t, J = 7.0, Ar-H), 7.33–7.45 (3H, m, Ar-H), 7.59 (1H, t, J = 7.0, Ar-H), 7.68 (1H, d, J = 8.2, Ar-H), 7.81 (1H, d, J = 7.6, Ar-H), 8.48 (2H, d, J = 7.9, Ar-H); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 26.7, 30.8, 41.2, 41.6, 114.0, 121.0, 125.2, 128.2, 128.4, 128.7, 130.0, 132.3, 139.2, 150.5, 159.7, 160.6; ESI-MS *m/z:* 293 $[M + H]^+$; found 293.1776 ($C_{18}H_{21}N_4$ $[M + H]^+$, requires 293.1766).

*6.1.3.23. 6-(2-Phenylquinazolin-4-ylamino)-hexanoic acid (**3w**)*. To a stirring solution of 4-chloro-2-phenylquinazoline **6** (119 mg, 0.49 mmol) in dioxane (4 ml) was added ε-amino-*n*-caproic acid **7w** (326 mg, 2.48 mmol) and sodium carbonate (267 mg, 2.52 mmol). At room temperature one solid was in suspension. The reaction vessel was fitted with a condenser and the mixture stirred at 98 °C for 94 hours. The reaction was followed by TLC and when complete the reaction mixture was dissolved in methanol (10 ml) to give a cloudy suspension. Acetic acid was added drop-wise until the precipitate dissolved. The solution was reduced under vacuum to yield an off-yellow solid which was recrystallised from ethyl acetate (20 ml) to give a white solid which was dissolved in methanol and toluene (11 ml, 10:1) and reduced to dryness, a procedure repeated twice. The solids were washed once more in methanol and toluene (11 ml, 10:1) and dried under high vacuum. 6-(2-Phenylquinazolin-4-ylamino)-hexanoic acid **3w** was isolated as a white crystalline solid (157 mg, 90%). IR (Solid): ν_{max} cm^{-1} 2939, 2860, 2360, 1570, 1531, 1406, 1322, 1221, 1126, 1013; ^1H NMR (250 MHz, d-MeOH): δ 1.50 (2H, q, J = 7.0, CH_2), 1.74 (2H, q, J = 7.6, CH_2), 1.87 (2H, t, J = 7.3, CH_2), 2.29 (2H, qu, J = 7.9, CH_2), 3.80 (2H, t, J = 7.0, CH_2), 7.48–7.56 (3H, m, Ar-H), 7.77 (1H, t, J = 8.2, Ar-H), 7.85 (1H, d, J = 8.2, Ar-H), 8.14 (2H, d, J = 8.2, Ar-H), 8.43 (2H, dd, J = 5.8, J = 2.1, Ar-H); ^{13}C NMR (62.5 MHz, d-MeOH): δ 25.4, 26.6, 28.4, 36.1, 40.6, 113.6, 121.8, 125.1, 126.3, 127.7, 128.0, 129.7, 132.3, 138.5, 149.2, 160.1, 160.9, 177.4; ESI-MS *m/z:* 336 $[M + H]^+$; found 336.1703 ($C_{20}H_{22}N_3O_2$ $[M + H]^+$, requires 336.1712).

*6.1.3.24. Methylphenyl-(2-phenylquinazolin-4-yl)-amine (**3x**)*. Conditions were as employed for **3a**. *N*-methylaniline **x** (212.6 mg, 1.98 mmol), **6** (245.9 mg, 1.02 mmol), reaction time 18 h, gave product **3x** (308.1 mg, 87%) as a solid. m.p., 256 °C; IR (Solid): ν_{max} cm^{-1} 2766.8, 1605.1, 1557.9, 1537.9, 1515.2, 1485.7, 1466.9, 1448.3, 1425.8, 1374.3, 1330.0, 1162.4, 1101.4, 1072.5; ^1H NMR (250 MHz, d^6-DMSO): δ 3.92 (3H, s, CH_3), 6.84 (1H, d, J = 8.5, Ar-H), 7.29 (1H, t, J = 8.1, Ar-H), 7.57–7.66 (6H, m, Ar-H), 7.70–7.92 (3H, m, Ar-H), 8.31 (1H, d, J = 8.2, Ar-H), 8.62 (1H, d, J = 8.2, Ar-H), 8.63 (1H, d, J = 7.9, Ar-H); ^{13}C NMR (62.5 MHz, d^6-DMSO): δ 43.6, 126.3, 127.4, 129.0, 129.2, 130.6, 134.7; ESI-MS *m/z:* 312 $[M + H]^+$; found 312.1494 ($C_{21}H_{18}N_3$ $[M + H]^+$, requires 312.1501).

6.2. Pharmacology

Evaluation of the activity and cytotoxicity of the compounds was performed in Scrapie-infected mouse brain (SMB) cell lines with exposure to accurate concentrations of candidate drug for 7 days. The assay was adapted from the methods of Rudyk et al. [27]. A persistently infected mouse cell line (SMB), cloned originally from scrapie infected mouse brain but of non-neuronal origin, was used for screening of compounds for their ability to prevent the formation of PrP^{Sc} [28]. The cells were grown in Medium 199 with Earle's salts (Containing 2.2 g l^{-1} sodium bicarbonate and glutamine as per formula) at 37 °C under 5% CO_2 in air at 95% relative humidity. The medium was supplemented with newborn calf serum (heat inactivated) 10%, foetal calf serum (heat inactivated) 5%, and penicillin-streptomycin at 10 mg l^{-1}. Every 7 days confluent cells were passaged using 0.05% trypsin, 0.002% EDTA, at a split ratio of 4:1. To assess the effects of compounds cells were distributed into 24-well cluster plates at 30 000 cells per well and incubated for 24 h to allow for cell attachment. The medium was changed for that dosed with the desired compounds which were prepared by dilution of a DMSO stock solution to the concentration required. The final DMSO concentration was at ≤ 0.2% (v/v). An equal concentration of DMSO was added to the medium of the untreated control. The exposures were carried out in duplicate on two plates to allow separate viability and activity measurements. Within each plate the experiments were carried out in triplicate.

6.2.1. Cytotoxicity assays in vitro

Viability of the cells following exposure was assessed using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT). MTT stock solution at 5 mg ml^{-1} was added to each well of the 24-well culture dish at 10% the original culture volume and incubated at 37 °C for 2 hours. After exposure the medium was removed and the formazan crystals formed

by mitochondrial dehydrogenase action were solubilised with acidic isopropanol (0.1 M HCl). Absorbance of the converted dye was measured at 570 nm with a background subtraction at 690 nm. The viability of the treated cells was measured as a percentage of untreated controls.

6.2.2. Dot-blot analysis of PrP^{Sc} levels

Cells were lysed with 0.1 ml lysis buffer (10 mM Tris–HCl pH 7.6, 100 nM NaCl, 10 mM EDTA, 0.5% (v/v) Nonidet®P 40 substitute, 0.5% (w/v) sodium deoxycholate) at 37 °C for 10 min. The contents of the triplicate wells for each specific treatment were combined and centrifuged at $1000 \times g$ for 5 min. The post-nuclear supernatant was removed and the total protein concentration per experiment was determined using a Bradford assay (Sigma). The supernatant was diluted accordingly to allow approximately 30–40 μg per 100 μl, which was loaded onto nitrocellulose membrane presoaked in lysis buffer under gentle vacuum (350 mmHg) via a 96-well dot-blot manifold. The membranes were allowed to air dry before treatment to discriminate PrP^{Sc} from PrP^{C}. Briefly, the membranes were immersed in 75 μg ml^{-1} proteinase K solution in Tris–HCl-buffered saline (TBS) for 60 min at 37 °C. The proteolysis was stopped with treatment with 1 mM phenyl-methyl-sulphonyl fluoride (PMSF) for 10 min. The PrP^{Sc} remaining was treated with 3 M guanidine thiocyanate to expose the epitopes, following which the membrane was blocked with 5% fat free milk powder in TBS. The dot-blot was then processed with 6H4 primary antibody (Prionics) prior to treatment with anti-mouse secondary antibody. The dot-blot was developed by enhanced chemiluminescence using the Western Breeze analysis kit (Amersham Biosciences). The image of the membrane was then captured using a light box and camera attached to a computer and the image was analysed using Labworks software.

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