Quinazolinone-based Anticancer Agents: Synthesis, Anti-proliferative SAR, Anti-tubulin Activity and Tubulin Co-crystal Structure

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Abstract

Quinazolinone-based anti-cancer agents were designed, decorated with functional groups from a 2-methoxyestradiol-based microtubule disruptor series, incorporating the aryl sulfamate motif of steroid sulfatase (STS) inhibitors. The steroidal AB-ring system was mimicked, favoring conformations with an *N*-2 substituent occupying D-ring space. Evaluation against breast and prostate tumor cell lines identified **7b** with DU-145 anti-proliferative activity (GI_{50} 300 nM). A preliminary structure-activity relationship afforded compounds (e.g. **7j** GI_{50} 50 nM) with activity exceeding that of the parent. Both **7b** and **7j** inhibit tubulin assembly *in vitro* and colchicine binding and **7j** was successfully co-crystallized with the αβ-tubulin heterodimer as the first of its class, its sulfamate group interacting positively at the colchicine binding site. Microtubule destabilization by **7j** likely prevents the curved-to-straight conformational transition. Quinazolinone sulfamates surprisingly showed weak STS inhibition. Preliminary *in vivo* studies in a multiple myeloma xenograft model for **7b** showed oral activity, confirming the promise of this template.

Introduction

In previous studies we optimized a series of sulfamoylated estratrienes based around 2-methoxyestradiol derivatives as anti-cancer agents.[1-6] These agents were developed as part of a programme addressing design of inhibitors of the emerging drug target steroid sulfatase (STS).[7] In particular, an aryl sulfamate pharmacophore motif imbued potent irreversible inhibition against STS and drugs bearing this motif entered clinical trials, primarily in oncology. The sulfamoylated estratrienes also showed potent STS inhibitory activity.[6] These also exhibit potent anti-proliferative activity against a range of human cancer

cell lines and also inhibit angiogenesis. This dual mechanism of action can be ascribed to inhibition of normal microtubule dynamics. In addition to good oral bioavailability and excellent *in vivo* activity,[7] they proved capable of inhibiting the growth of cell lines resistant to existing microtubule disruptors such as the taxanes. To develop further series of compounds sharing this mechanism of action, we were drawn to investigate whether, by translating the three key pharmacophore elements from a sulfamate steroidal compound series **1** (*eg* **1a** (STX140), **Figure 1**) into non-steroidal motifs, we could generate new microtubule disruptors with enhanced activity and particularly more useful physicochemical properties. In initial studies,[8-9] we used a tetrahydroisoquinoline (THIQ) decorated at C-6 and C-7 to mimic the steroidal AB-rings, tethered through *N*-2 to a D-ring mimic, initially a benzyl group. This projects a hydrogen bond acceptor into the appropriate region of space to address the pharmacophore for anti-proliferative activity in that region. This preliminary work delivered a series of microtubule disruptors with anti-proliferative activity in the micromolar range, demonstrating also enhanced activity for the sulfamoylated phenols, and this activity could be further optimized by introducing a substituent at *C*-3 to sterically inhibit the free rotation of the *N*-benzyl group. This manoeuver favored a conformational population in which the *N*-benzyl group is in proximity to the steroidal D-ring; in this manner compounds displaying nanomolar activity (equivalent to the steroidal derivatives upon which their design was based) were elaborated.[10] In tandem, related chimeric microtubule disruptors built from the THIQ core and the trimethoxy aryl motif common to many colchicine site binders on tubulin were constructed.[11-12]

In the present work, we aimed to utilize a similar strategy for the discovery of new microtubule disruptors, but extend our previous efforts by incorporating an achiral steric buttress to achieve conformational biasing. Having earlier successfully mimicked the steroidal AB-ring system with the 6,6-bicyclic THIQ core and achieved enhanced activity by substituting at *C*-3, with concomitant introduction of chirality, we were drawn to construct 3,4-dihydroquinazolin-2(*1H*)-one (DHQ)-based candidates and their sulfamate derivatives (**Figure 1**).

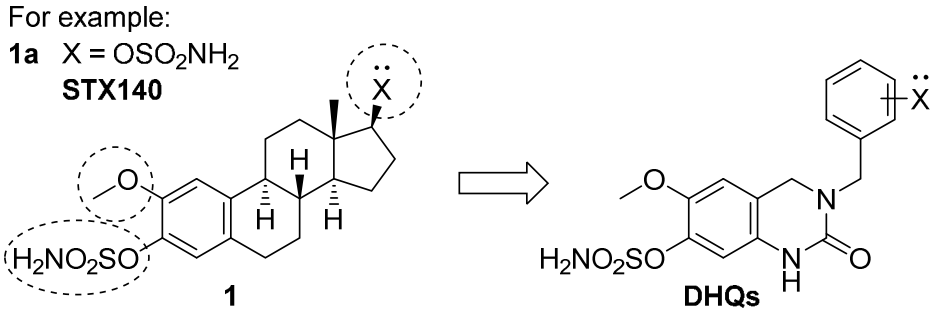


Figure 1: Design of quinazolinone-based microtubule disruptors (DHQs) from the steroidal three-point pharmacophore. X: is a H-bond acceptor.

We reasoned that the carbonyl group at *C*-3 should clash with the *N*-2 substituent that would then prefer to adopt a conformation in which it projects into the area of space close to that of a mimicking steroidal D-ring. This would place a hydrogen bond acceptor group at a near optimal position for target site interaction. By introducing 2'-, 3'- and 4'-methoxybenzyl groups at *N*-2 we envisaged that prototypical hits could be obtained. Should such hits be identified, a small screening set could be constructed to afford a preliminary SAR.

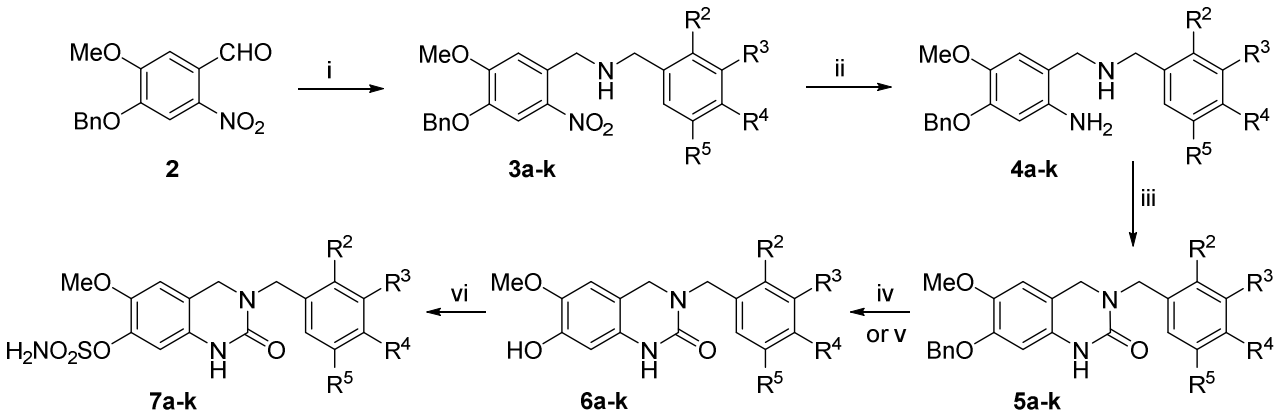
Results and Discussion

Chemistry

The candidate DHQs were duly synthesized in a five step linear synthesis for each sulfamate derivative of type **7** starting from 4-benzyloxy-5-methoxy-2-nitrobenzaldehyde **2**.[13] Reductive amination of **2** with the corresponding benzylamine in the presence of sodium triacetoxyborohydride in chloroform gave compounds **3a-k**. Subsequent reduction of the nitro group using Raney nickel and hydrazine hydrate in methanol furnished the corresponding anilines **4a-k** in very good yields. Reaction of **4a-k** with urea at 220 °C delivered the protected quinazolinones **5a-k**. These were transformed into the unprotected phenols **6a-k** either by treatment with hydrogen and palladium on carbon (Pd/C) or with

methanesulfonic acid in dichloromethane (for **5f**) and finally into the target sulfamates **7a-k** using sulfamoyl chloride in *N,N*-dimethylacetamide (DMA)[14] (**Scheme 1**).

Scheme 1[a] Synthesis of quinazolinone-based microtubule disruptors.



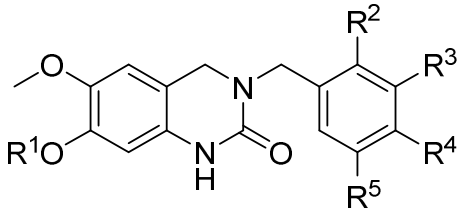
[a]*Reagents and conditions*: i) $ArCH_2NH_2$, $NaB(OAc)_3H$, $CHCl_3$, reflux; ii) Raney Ni, $N_2H_4 \cdot H_2O$, MeOH, reflux; iii) Urea, 220 °C; iv) H_2, Pd/C, THF/MeOH, rt; v) CH_3SO_3H, CH_2Cl_2, rt; vi) H_2NSO_2Cl, DMA, rt.

Biology

The *in vitro* activities of the DHQs and their sulfamates against the proliferation of DU-145 prostate and MDA MB-231 breast cancer cell lines were determined and are presented in **Table 1** and are benchmarked against literature data for combretastatin A-4 (**CA-4**). Data obtained for both cell lines are in good agreement, and therefore, for clarity of SAR discussion, only the figures obtained with the DU-145 cells are discussed further. Compounds **6a** and **7a**, both of which lack a hydrogen bond acceptor and thus one component of the steroidal pharmacophore for microtubule disruption, proved inactive, as expected. The results obtained for the simple methoxybenzyl DHQs were more encouraging. While the 4'-methoxy and 3'-methoxy derivatives showed at best modest activity (**7c** GI_{50} 33 μM), the 2'-methoxy derivative proved extremely interesting, with the 6-hydroxy compound and its 6-*O*-sulfamate,

respectively, exhibiting micromolar and nanomolar GI_{50}'s. This finding, that sulfamate **7b** was more active than **6b**, is in agreement with the steroidal series, wherein sulfamoylation of the phenol typically results in a 10-fold or greater increase in activity. In this case, and for later members of the DHQ series, this rule of thumb holds well (GI_{50}'s of 0.3 μM and 2.2 μM for **7b** and **6b** respectively). These preliminary results indicated that 2'-substitution was worthy of further investigation, and a second set of phenols **6e-k** and their sulfamates **7e-k** was elaborated. Groups of similar steric size but differing electronic properties were introduced at the 2'-position, the lipophilic methyl group afforded little gain of activity, while the small fluoro substituent improved activity only modestly (**7g** GI_{50} 4.8 μM). In strong contrast, the 2'-chloro derivative **7f**, which offers unhindered H-bond acceptor potential, showed excellent activity (GI_{50} 0.5 μM), thus providing support for our working hypothesis that decorating a non-steroidal system with appropriate functionality and arrangement to satisfy the established steroidal pharmacophore should yield novel microtubule disruptors. The 3'-chloro compound **7h** proved significantly more active (*ca* 10-fold) than the corresponding 3'-methoxy compound **7c**, suggesting that 3'-substitution was not necessarily deleterious to activity and also confirming that 2'-substitution is highly favored.

Table 1. Anti-proliferative activity of DHQs against DU-145 human prostate and MDA MB-231 human breast cancer cells *in vitro*.[a]



compd	R^1	R^2	R^3	R^4	R^5	GI_{50} (μM) DU-145	GI_{50} (μM) MDA MB-231
6a	H	H	H	H	H	>100	>100
7a	SO_2NH_2	H	H	H	H	>100	>100

6b	H	OMe	H	H	H	2.2	5.7
7b	SO_2NH_2	OMe	H	H	H	0.3	0.2
6c	H	H	OMe	H	H	>100	>100
7c	SO_2NH_2	H	OMe	H	H	33	34
6d	H	H	H	OMe	H	>100	>100
7d	SO_2NH_2	H	H	OMe	H	>100	>100
6e	H	Me	H	H	H	>100	>100
7e	SO_2NH_2	Me	H	H	H	60	7.4
6f	H	Cl	H	H	H	5.6	5
7f	SO_2NH_2	Cl	H	H	H	0.5	0.2
6g	H	F	H	H	H	>100	>100
7g	SO_2NH_2	F	H	H	H	4.8	4.5
6h	H	H	Cl	H	H	81	>100
7h	SO_2NH_2	H	Cl	H	H	3.2	2.2
6i	H	OMe	OMe	H	H	8.5	8.3
7i	SO_2NH_2	OMe	OMe	H	H	0.8	0.4
6j	H	OMe	H	H	OMe	1.5	2.3
7j	SO_2NH_2	OMe	H	H	OMe	0.05	0.05
6k	H	H	OMe	OMe	OMe	8.4	9.3
7k	SO_2NH_2	H	OMe	OMe	OMe	0.1	0.1
CA-4	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]	0.27[c]	0.045[c]

[a] GI_{50} figures are the mean values obtained from experiments performed in triplicate. [b] NA: Not applicable. [c] GI_{50} values are taken from the literature.[15]

Two dimethoxy analogues were also evaluated, with 2',3'- and 2',5'-substitution being selected on the grounds that the inductive effect of a second methoxy group might increase the electron density on the 2'-methoxy and thus enhance H-bonding. The 2',3'-dimethoxy compound **7i** proved less active than **7b**, suggesting that the additional methoxy at the 3'-position hinders binding, presumably on steric grounds. The 2',5'-dimethoxy compound **7j** (GI_{50} 50 nM), in contrast, was 6-fold more active than **7b**, indicating that enhanced hydrogen bonding may have resulted from the increased electron density on the 2'-methoxy derivative, although additional electrostatic or lipophilic interactions at the 5'-position could equally underlie this improvement in activity. The final compound to be evaluated was the 3',4',5'-trimethoxy compound **7k**, which also displayed excellent activity (GI_{50} 0.1 μM). We selected this tri-substituted motif for further evaluation, since the trimethoxyphenyl moiety is common to many colchicine site binding natural products and we had observed in our earlier work in the THIQ series

strong activity for compounds bearing this motif.[11] In that case, however, the SAR varied from that of our steroidomimetic THIQs, and we considered that such derivatives should be considered chimeras of both our steroidal microtubule disruptors and trimethoxyaryl colchicine site binders. Whether **7k** can be considered a chimeric microtubule disruptor is an open question, and further studies will be required for a definitive answer. In any case, it is a welcome additional lead from our comparatively small discovery compound set.

With these excellent *in vitro* data in hand, we then established the microtubule disruptor activity of compounds **7b, 7f** and **7i-k** alongside the established potent microtubule disruptor **CA-4** (**Table 2**). The 2′-methoxy and 2′,5′-dimethoxy derivatives **7b** and **7j** both inhibit tubulin assembly very effectively with an IC_{50} of 2.5 μM and are only 2- to 3-fold less active than **CA-4** itself. The concentration required in tubulin-based assays greatly exceeds the anti-proliferative dose; most likely it suffices to disrupt microtubule dynamics to arrest the cell cycle rather than causing a catastrophic depolymerization event. Also, the nominal compound concentration in anti-proliferative assays is that of agent added to the culture medium, rather than the actual concentration within cells. We also determined that **7b**, **7f** and **7j** inhibit colchicine binding to tubulin, with **7j** being the best, showing 61% inhibition at 5 μM. It thus appears reasonable to suggest that the interaction of the novel quinazolinones can at least partially be ascribed to their ability to disrupt the normal dynamic polymerization of tubulin by interaction at the colchicine binding site. We thus chose to explore this idea further using structural biology techniques.

Table 2. Activity of selected quinazolinones as inhibitors of tubulin polymerization and [^{3}H]colchicine binding (5 μM inhibitor) to tubulin.[a]

compd	Tubulin assembly IC_{50} (μM)	Colchicine binding (% Inhibition)
CA-4	1.1 ± 0.1	99 ± 0.6
7b	2.5 ± 0.4	55 ± 2
7f	5.0 ± 0.7	43 ± 0.09
7i	8.6 ± 0.4	ND[b]

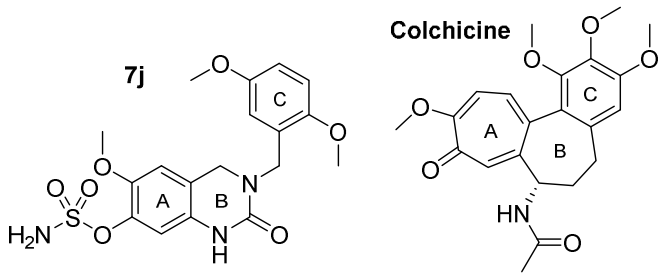
7j	2.5 ± 0.1	61 ± 2
7k	5.6 ± 0.02	ND[b]

[a] Values are the mean ±SD of at least two determinations. [b] ND: Not determined.

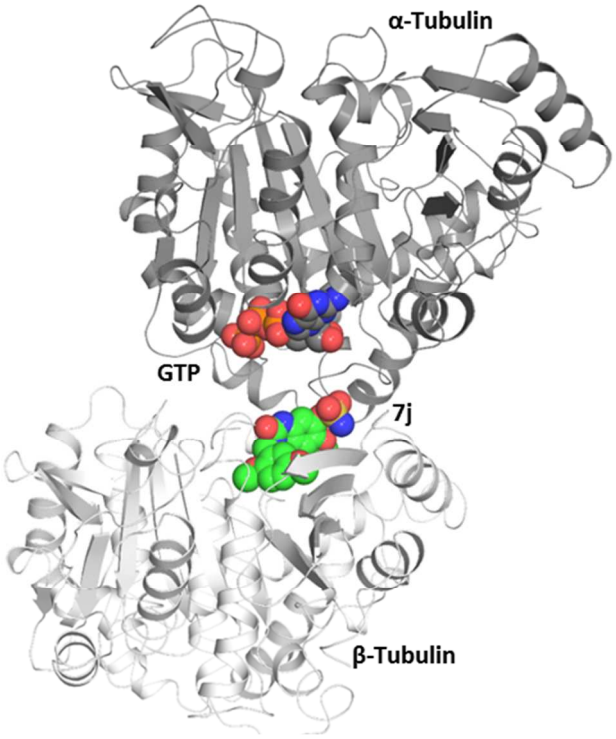
In order to determine the binding mode of these DHQ derivatives (**Figure 2a**) within the αβ-tubulin heterodimer, we soaked the most potent compound **7j** into a T_2R-TTL crystal[16] and solved the T_2R-TTL-**7j** complex structure by X-ray crystallography to 2.1 Å resolution (**Figures 2b**, **3a**; **Table S1** see also Supplementary Information). **7j** binds to the colchicine site of tubulin[17] at the intradimer interface and interacts with residues from the strands βS8 and βS9 and helix βH8 of β-tubulin and from the loop αT5 of α-tubulin (**Figure 3a**). A molecule of GTP was also observed bound to the α-subunit. As anticipated from the design considerations **7j** presents in an extended "steroid-like" conformation. The compound is well accommodated within the colchicine site, which undergoes significant conformational changes located at both the βT7 and αT5 loops of tubulin upon ligand binding (**Figure 4**). To the best of our knowledge this is the first co-crystal structure of an anti-tubulin agent possessing a sulfamate group. Sulfamoylation is known to enhance pharmaceutical properties and thus may be worth incorporating into other anti-tubulin agents binding to the colchicine site.

Figure 2: Crystal structure of the tubulin-**7j** complex. a) Structures of **7j** and colchicine. b) Binding mode of **7j** within the αβ-tubulin heterodimer (PDB ID 5OSK). The α- and β-tubulin subunits are shown in dark and light gray ribbon representation, respectively. The carbon atoms of **7j** and GTP are shown as green and orange spheres, respectively.

(a)



(b)

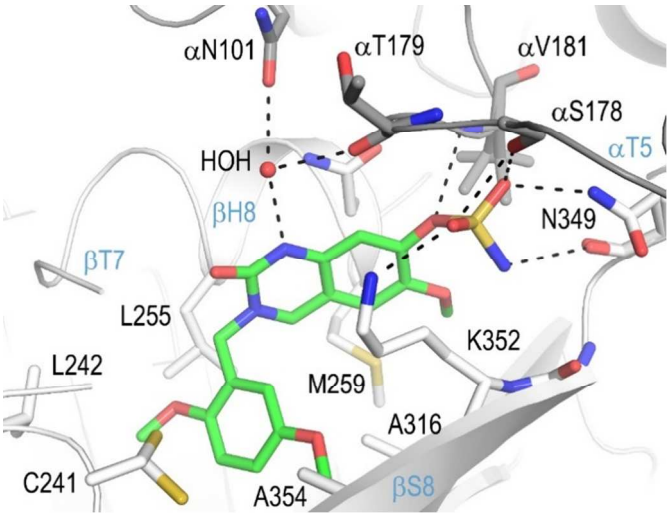


The compound is anchored into a hydrophobic pocket which is part of the β-tubulin subunit and formed by residues βLeu242, βLeu255, βCys241, βMet259, βAla316 and βAla354 (**Figure 3a**). Its interaction is further stabilized by hydrogen bonds mediating contacts mainly between the sulfamate group and the side chains of βLys352, βAsn349 and αSer178, the amine group of the main chain of αVal181 and the carbonyl group of the main chain of βAsn349. A supplementary water-mediated interaction (HOH) is formed between the amine group of the ligand's B ring and the carbonyl groups of the side chain and main chain of αAsn101 and αThr179, respectively (**Figures 3a** and **3b**). Given this apparently available space, further medicinal chemistry development of these DHQs could include attempting to mimic at least one of these interactions by substitution on the NH of the B ring with suitably-sized substituents (*eg N*-methylenehydroxy, *N*-methyleneamino and *N*-sulfonamide groups).

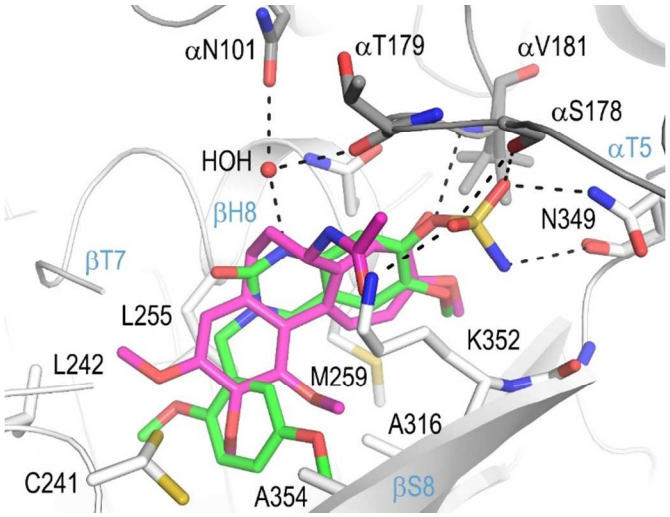
Figure 3: Detailed interaction of the tubulin-**7j** complex. a) Close-up view of the interaction mode between **7j** and tubulin (PDB ID 5OSK). Carbon atoms of **7j** and interacting residues are shown in

green and grey sticks, respectively. Oxygen, nitrogen and sulfur atoms are colored in red, blue and yellow, respectively. Hydrogen bonds are represented with black broken lines. Secondary structural elements are labelled in blue. The βT7 loop has been truncated due to the flexibility within the crystal upon **7j** ligand binding. b) Superimposition of the tubulin-**7j** (carbon atoms as green sticks) and the tubulin-colchicine (PDB ID 4O2B; carbon atoms as magenta sticks) complex structures.

(a)



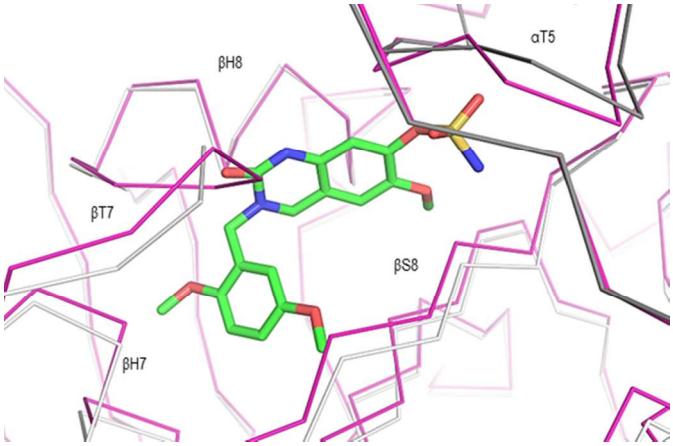
(b)



We then sought to compare the binding mode of **7j** with that of colchicine and superimposed both β-tubulin chains of the respective tubulin-ligand complexes ($RMSD_{chainD\ colchicine\ onto\ chainD\ 7j}$ 0.4 Å over 2808 atoms; **Figures 3a**, **3b**, **4**; PDB ID 4O2B). Both compound poses are similar and reveal that the

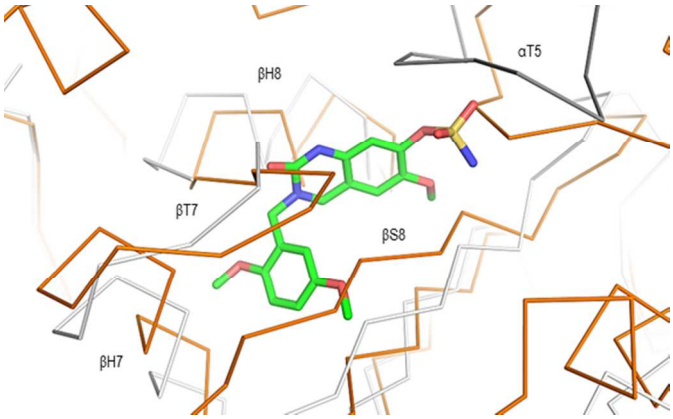
respective A rings and associated methoxy groups of **7j** and colchicine superimpose very well. We found that **7j** is more deeply inserted into the binding pocket than colchicine and that both compounds share a unique common polar interaction with the amine group of the main chain of αVal181.

Figure 4: Superimposition of the apo and **7j**-bound tubulin structures. The β2-tubulin chain of the T_2R-TTL apo complex (PDB ID 4I55; magenta ribbon) is superimposed onto the respective β2-tubulin chain of the tubulin-**7j** structure (dark and light grey ribbon representation for α- and β-tubulin, respectively) and secondary structure elements are labeled.



Free tubulin undergoes a characteristic "curved-to-straight" conformational change upon polymerization into microtubules.[17-18] This conformational change involves an overall compaction of the colchicine binding site involving mainly the βT7 loop and βS8 strand of the β-tubulin chain.[17] To investigate the binding of **7j** in the context of a microtubule, we superimposed both β-tubulin chains from a "straight" tubulin structure (PDB ID 3JAR) and our tubulin-**7j** structure ($RMSD_{\text{chainD "straight" onto chainD 7j}}$ 0.7 Å over 1609 atoms; **Figure 5**). We observe that upon binding to free tubulin, compound **7j** prevents the compaction of the colchicine site, by sterically hindering the accommodation of the βT7 loop and βS8 strand that occlude the colchicine binding site. Presumably, the extra interactions of the **7j** sulfamate group *vs* colchicine itself further strengthens this effect. Thus, **7j** is expected not to be able to bind to the colchicine sites in preformed microtubules.

Figure 5: Binding of **7j** in the context of a microtubule. The *N*- and *C*-terminal domains of β-tubulin from a "straight" tubulin structure (PDB ID 3JAR; orange ribbon) have been superimposed onto the corresponding domains of "curved" β-tubulin from the T_2R-TTL-**7j** structure (light and dark grey ribbons).



In summary, these results establish compound **7j** as a colchicine site tubulin binding ligand. They further suggest that, similar to other colchicine site ligands,[17, 19] the mechanism of microtubule destabilization by **7j** is to prevent the curved-to-straight tubulin conformational transition.

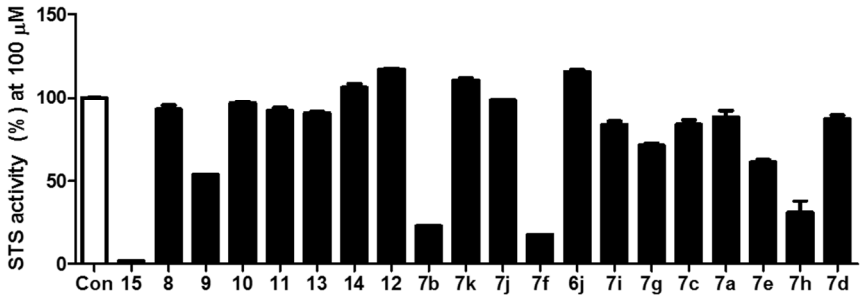
All sulfamoylated compounds of type **7** and one phenol of type **6** were also tested for steroid sulfatase (STS) inhibition (structures see also **S4** in SI). STS Inhibition is a new strategy for endocrine therapy,[7] as for the well-established aromatase inhibition. As a comparison, some previously synthesized tetrahydroisoquinoline derivatives **8-14**[9, 11-12] and the well-known potent inhibitor irosustat **15**[7, 20] (all structures see **S5** in SI) were also evaluated alongside the quinazolinone derivatives, with the expectation that, like most aryl sulfamate esters,[6-7] they would show good STS inhibitory activity.

However, at 10 μM only compounds **7b**, **7f** and **7h** showed weak activity (see **S6a** in SI). At 100 μM, however, the same three compounds inhibited STS by about 70-80% (**Figure 6a**), with other compounds also starting to show weak activity. It seems that substitution in the 2'-position of the C-ring

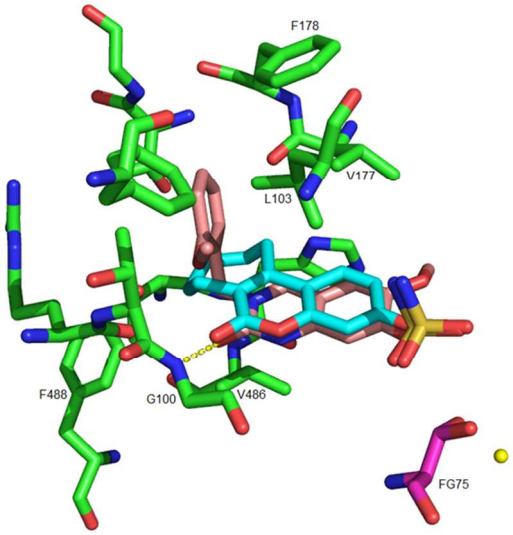
of the DHQs is favorable to obtain weak STS inhibition, with **7f** (2'-Cl) and **7b** (2'-MeO) being about equally active. Compounds with other substituents in the 2-position, like **7e** (2'-Me) and **7g** (2'-F), are weakly active, with an inhibition of about 20-25% at 100 μM. At the 3'-position of the C-ring, only some small H-bond acceptors like Cl are tolerated (**7h**), in contrast to MeO (**7c**), which is not. It seems though that overall there is still some H-bond acceptor property required in the C-ring to obtain at least some STS inhibition, as the all H-substituted compound **7a** proved inactive. All di- or tri-substituted compounds of type **7** and **6j** were also inactive even at the higher 100 μM concentration (**Figure 6a**). Remarkably, this also included compounds **7j** and **7k**, which showed excellent *in vitro* anti-proliferative activity against both cancer cell lines. Of the earlier non-steroidal tetrahydroisoquinoline (THIQ) derivatives **8-14** evaluated, only compound **9** showed some weak STS inhibition (about 45% at 100 μM). Irosustat **15** was used as a positive control and was, as expected, highly potent, with near total STS inhibition at both concentrations.

Figure 6: a) Inhibition of steroid sulfatase by DHQ and THIQ derivatives at 100 μM inhibitor concentration. b) Docking of **7b** (pink) and irosustat **15** (cyan) into the crystal structure of human STS. The Ca^{2+} ion is depicted as a yellow sphere, and FG75 is the gem-diol form of FGly 75 aldehyde. Dotted line is a potential hydrogen bond.

(a)



(b)



The weak activity towards STS of both DHQ and THIQ classes of compound was unexpected, since generally aryl sulfamate esters are highly active on STS.[7] Docking studies were conducted to explore potential interactions between the quinazolinone derivatives and the STS active site, in a similar fashion to those carried out for STX64/irosustat and related series members.[20] **7b** Is placed in a very similar fashion to the irreversible STS inhibitor irosustat, with the sulfamoyl group in close proximity and opposed to the catalytic FGly 75 (**Figure 6b**), suggesting that a putative sulfamoyl group transfer could also occur leading to similar irreversible inhibition. The bicyclic system is sandwiched between V486 underneath the rings, and L103 and V177 above the rings. **7b** Forms a possible hydrogen bond with the NH of G100 via their quinazolinone oxygen (N…O = ~3.3 Å) in the same manner as **15** does via its lactone oxygen (**Figure 6b**). However, compared with irosustat, it may be that the flexibility of these DHQ- and THIQ-based compounds prevents them easily reaching deep into the STS binding pocket. The modeling results do not, however, suggest any obvious reasons to explain the modest activity nor anything that might clash with the positive charge on the THIQ derivatives (data not shown), as these compounds dock very well into the active site. Similarly to the antitubulin activity, DHQ derivatives do possess some potential to be further optimized to improve STS inhibitory activity. **7b** and **7f** are overlaid in the STS binding site (see **S6b** in SI). Both DHQ and THIQ sulfamates might also merit

exploration and development as CAIX inhibitors, as sulfamate esters are known to imbue CAIX inhibitory and modulatory activities,[21] and this is an emerging area of clinical interest.[22]

On the basis of its promising *in vitro* activity, **7b** was selected as an early lead for a preliminary evaluation in an RPMI 8226 *in vivo* xenograft model of multiple myeloma (see **S7a** and **S7b** in SI). The compound was dosed alongside vehicle at 20 mg/kg and 40 mg/kg orally for 28 days (the standard doses and regime used for the earlier steroidal and non-steroidal systems explored).[9] A statistically significant reduction in tumor growth of 27% and 31%, respectively, was observed at the two doses at day 53 (18 days after cessation of treatment). Future studies, preferably with **7j** or a more active compound should ideally be preceded by pharmacokinetic evaluation. Importantly, this non-steroidal system was clearly well-tolerated *in vivo*, with the sulfamate moiety likely contributing to oral bioavailability.

Microtubule-targeting agents have a long history of clinical efficacy, remain the most classical and reliable antimitotics and disrupt proper microtubule dynamics. So far, novel antimitotic agents have shown limited efficacy in clinical trials and classical antimicrotubule drugs are still the best approach in targeting mitosis. However, despite the clinical success of *eg* paclitaxel, treating hormone-refractory breast cancer remains challenging, and the drug has a poor pharmacological profile accompanied by severe dose limiting toxicities, such as neutropenia and peripheral neuropathy. Work on the rational design of, and search for, new antimitotic compounds is therefore still highly justifiable. We have pioneered the application of sulfamate ester-based drugs in anticancer drug design.[7, 23] Sulfamoylation provides attractive pharmaceutical and pharmacodynamic advantages, as well as conferring oral activity,[24] something much less common in antimitotic drugs. For example, one agent of promise is the steroid *bis*-sulfamate **1a** (STX140).[25] STX140 effectively blocked the development of tumors in several models of breast cancer and significantly inhibited growth of those that did develop. Animals in early and late stage intervention groups saw a significant survival advantage. Moreover, unlike with paclitaxel no metastasis was observed, and this may be linked to the interaction of the sulfamate moiety with the hypoxic tumor target CAIX. Furthermore, unlike paclitaxel, STX140 did not induce significant

peripheral neuropathy and neutropenia, which are often dose-limiting. Additionally, we have shown the efficacy of non-steroidal derivatives of STX140[12] in xenograft models *in vivo*[26] and more widely[27] and that such drug candidates interact with tubulin and can be active in models of clinical taxane resistance.[26] While it has yet to be shown more widely that such non-steroidal sulfamate esters confer all of the above advantages shown by STX140, it is notable that the present work reports the first example of such an agent in atomic detail bound to tubulin dimer, as determined by structural biology techniques. It is highly encouraging that the sulfamate group is not only well-accommodated, but also makes positive interactions in the colchicine site. Colchicine itself has often been linked to adverse toxicity, and there is currently no colchicine site microtubule disruptor approved in oncology, although agents are, and have been, in clinical trials. Compound **7j** and similar quinazolinone sulfamates may thus be promising leads as novel antitubulin agents with desirable physicochemical and biological properties for oncology. The atomic level detail of the tubulin-**7j** structure reported here, moreover, shows opportunities for application of medicinal chemistry to improve interactions further with the colchicine site. Taken together, these results justify the wider exploration of these quinazolinones and of sulfamate-based agents in oncology.

Conclusions

Quinazolinone-based microtubule disruptors with excellent *in vitro* activities combined with desirable drug-like profiles have been designed. The best compound **7j** shows anti-proliferative activity in the 50 nM range, inhibits tubulin assembly and also interferes effectively with the colchicine site. **7j** was successfully co-crystallized with the αβ-tubulin heterodimer and found to bind more deeply in the colchicine binding site than colchicine itself, with the sulfamate group involved in the binding process through specific interactions with β-tubulin. This is the first example of a sulfamate ester bound to tubulin explored crystallographically. The results suggest that the mechanism of microtubule destabilization by **7j** is to prevent the curved-to-straight tubulin conformational transition that is

required for the formation of microtubules. Compound **7b** was evaluated positively for *in vivo* activity in a mouse xenograft model of multiple myeloma, confirming the promise of this new class of compounds. DHQ sulfamate derivatives also inhibit steroid sulfatase, but to date are only of moderate potency. However, DHQ sulfamates merit further optimization and exploration as anticancer agents and the preliminary *in vivo* study confirmed excellent tolerability. DHQ and THIQ sulfamate compound classes might also merit exploration and development as CAIX inhibitors. The crystallographic understanding of the DHQ sulfamate derivatives with tubulin reported here, in tandem with the highly favorable pharmaceutical properties afforded by sulfamate esters, should stimulate the design of other orally active colchicine site binding microtubule disruptors.

Experimental Section

Biology. In Vitro Studies: Cell Lines. DU145 (brain metastasis carcinoma of the prostate) and MDA-MB-231 (Metastatic pleural effusion of breast adenocarcinoma) established human cell lines were obtained from ATCC Global Bioresource Center. Cells were maintained in a 5% CO_2 humidified atmosphere at 37 °C in RPMI-1640 medium, supplemented with 10% fetal bovine serum, penicillin, and streptomycin.

Anti-proliferative Assays. DU145 and MDA-MB-231 cells were seeded into 96-well microtiter plates (5000 cells/well) and treated with 10^{-9}-10^{-4} M of compounds or with vehicle control. At 96 h post-treatment, live cell counts were determined by WST-1 cell proliferation assay (Roche, Penzberg, Germany), as instructed by the manufacturer. Viability results were expressed as a percentage of mean control values resulting in the calculation of the 50% growth inhibition (GI_{50}). All experiments were performed in triplicate.

Tubulin Assays. Bovine brain tubulin, prepared as described previously,[28] was used in the studies presented here. Assembly IC_{50}'s were determined as described in detail elsewhere.[29] Briefly, 1.0 mg/mL

(10 μM) tubulin was preincubated without GTP with varying compound concentrations for 15 min at 30 °C. Reaction mixtures were placed on ice, and GTP (final concentration, 0.4 mM) was added. The reaction mixtures were transferred to cuvettes, held at 0 °C in a recording spectrophotometer. Baselines were established at 0 °C, and increase in turbidity was followed for 20 min following a rapid (< 30 s) jump to 30 °C. Compound concentrations required to reduce the turbidity increase by 50% were determined. The method for measuring inhibition of the binding of [^{3}H]colchicine to tubulin was described in detail previously.[30] Reaction mixtures contained 0.1 mg/mL (1.0 μM) tubulin, 5.0 μM [^{3}H]colchicine, and potential inhibitor at 5.0 μM. Compounds were compared to CA-4, a particularly potent inhibitor of the binding of colchicine to tubulin.[31] Reaction mixtures were incubated for 10 min at 37 °C, a time point at which the binding of colchicine in control reaction mixtures is generally 40-60% complete.

Crystallization, Data Collection and Structure Solution. Crystals of T_2R-TTL were grown as previously described[16, 32] and soaked for 3 h at 20 °C in the reservoir solution (10% PEG 4k, 16% glycerol, 30 mM $MgCl_2$, 30 mM $CaCl_2$, and 100 mM 2-(*N*-morpholino)ethanesulfonic acid/imidazole (pH 6.7)) containing 5 mM of compound **7j**. Crystals were fished from the drop, transferred into cryo-solution (10% PEG 4k, 20% glycerol) containing 5 mM of compound **7j** and flash-cooled in a nitrogen stream at the beamline. Standard data collection at beamline x06SA at the Swiss Light Source (Paul Scherrer Institut, Villigen, Switzerland), data processing, and structure solution using the difference Fourier method were performed as previously described.[16, 32] Data collection and refinement statistics are given in **Table S1** (see Supplementary Information). The atomic coordinates and structure factors have been deposited in the Protein Data Bank (www.rcsb.org). The PDB access code for T_2R-TTL-**7j** is 5OSK.

Structural Analysis and Figure Preparation. Figures were prepared using the PyMOL Molecular Graphics System, version 1.7.6.1 (Schrödinger, LLC). Chains in the T_2R-TTL complex were defined as

follows: chain A, α1-tubulin; chain B, β1-tubulin; chain C, α2-tubulin; chain D, β2-tubulin; Chains A and B were used throughout for the structural analyses and figure preparation.

In Vitro Sulfatase Assay: STS activity was measured as previously described.[33] Briefly, JEG-3 cells were lysed with RIPA buffer (Sigma-Aldrich, U.K.) and protein content determined using a BCA assay (Thermo Fisher Scientific, UK). JEG-3 cells are known to possess high STS activity. From this lysate, 50 μg of cell protein with or without inhibitors was incubated for 3 h in PBS containing [6,7-^{3}H] E1S (4 × 10^{5} dpm, Perkin-ElmerLS, Boston, MA, USA) adjusted to a final concentration of 20 μM with unlabeled E1S. [4-^{14}C] E1 (1 × 10^{4} dpm, Perkin-Elmer) was used to monitor procedural losses. E1 (estrone) was separated from E1S (estrone sulfate) by partition with toluene and ^{3}H and ^{14}C radioactivity measured by liquid scintillation spectrometry. Resultant conversion of E1S to E1 was determined as pmol E1 formed/h/mg protein, and results show percentage of STS activity inhibited by compounds.

In Vivo Studies: Female NCr-nude mice, 4-6 weeks of age (acquired from Harlan Labs), were fed water *ad libitum* and an autoclaved standard rodent diet consisting of 18% protein, 5% fat, 5% fiber, 8% ash, and 3% minerals. Mice were housed in isolators on a 12-hour light-dark cycle at 22 °C and 40%-60% humidity. Animal care was in accordance with institutional guidelines. Tumor cells (6 x 10^{6} cells/animal) were implanted subcutaneously into the left flank of mice. Multiple myeloma cancer cells were implanted with an equal volume of Matrigel to increase take rate. Tumors were monitored initially twice weekly, and then daily as the neoplasms reached the desired size, approximately 100 mm^{3} (100 mg). When the tumors attained this predetermined size, the animals were randomized into 3 groups with 6 animals per group. Estimated tumor weight was calculated using this formula: Tumor Weight (mg) = (w^{2} x l)/2 where w = width and l = length in mm of the multiple myeloma tumor.

Molecular Modeling: All ligands were built and minimized using Schrödinger software running under Maestro version 9.0. The crystal structure of human placental estrone/DHEA sulfatase (PDB ID 1P49)[34] was used for building the gem-diol form of STS. This involved a point mutation of the ALS75 residue

in the crystal structure to the gem-diol form of the structure using editing tools within the Schrödinger software. The resulting structure was then minimized with the backbone atoms fixed to allow the *gem*-diol and surrounding side chain atoms to adopt low-energy confirmations. GOLD was used to dock the ligands 25 times each into the rigid protein, with the binding site being defined as a 10 Å sphere around the ALS75 sulfate. The docked poses were scored using the GOLDScore fitness function.

Chemistry. All chemicals were either purchased from Aldrich Chemical Co. (Gillingham, UK) or Alfa Aesar (Heysham, UK). Organic solvents of A.R. grade were supplied by Fisher Scientific (Loughborough, UK) and used as supplied. The petroleum ether (PE) used for column chromatography was of fractions 40-60 °C. $CHCl_3$, CH_2Cl_2, *N,N*-dimethylacetamide (DMA), *N,N*-dimethylformamide (DMF) and tetrahydrofuran (THF) were purchased from Aldrich and stored under a positive pressure of N_2 after use. Sulfamoyl chloride was prepared by an adaptation of the method of Appel and Berger[35] and was stored in the refrigerator under positive pressure of N_2 as a solution in toluene as described by Woo et al.[36] An appropriate volume of this solution was freshly concentrated in vacuo immediately before use. Compound **2** was prepared according to literature procedure.[13] Reactions were carried out at room temperature unless stated otherwise. Flash column chromatography was performed on silica gel (MatrexC60). ^{1}H NMR and ^{13}C NMR spectra were recorded with either a JMN-GX 270 at 270 and 67.5 MHz, respectively, or a Varian Mercury VX 400 NMR spectrometer at 400 and 100 MHz, respectively (^{13}C NMR data see **S2** in SI). Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane as internal standard. Mass spectra were recorded at the Mass Spectrometry Service Centre, University of Bath, UK. FAB-MS were carried out using *m*-nitrobenzyl alcohol (NBA) as the matrix. Elemental analyses (results see **S3** in SI) were performed by the Microanalysis Service, University of Bath. Melting points were determined using a Stanford Research Systems Optimelt MPA100 melting point apparatus (Stanford Research Systems, Sunnyvale, CA, USA) and are uncorrected. All compounds were ≥96% pure by reversed-phase HPLC run with CH_3CN/H_2O or $MeOH/H_2O$ (Sunfire C18 reversed-phase column, 4.6 x 150 mm, 3.5 μm pore size) and are uncorrected.

N-(4-Benzyloxy-5-methoxy-2-nitrobenzyl)-(phenyl)-methanamine **3a**

Compound **2** (2.87 g, 10.0 mmol), benzyl amine (1.32 mL, 12.0 mmol) and sodium triacetoxyborohydride (3.0 g, 14.0 mmol) were dissolved in $CHCl_3$ (50 mL) and refluxed for 8 h and then cooled to rt. The reaction mixture was diluted with $CHCl_3$ (50 mL), washed with $NaHCO_3$ (sat., 80 mL), water and brine, dried ($MgSO_4$), filtered and concentrated in vacuo. Flash column chromatography (PE/EtOAc 20:1→1:3) afforded compound **3a** as a yellow powder (2.5 g, 66%); mp 107-109 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.96 (1H, s, br), 3.83 (2H, s), 3.95 (3H, s), 4.06 (2H, s), 5.17 (2H, s), 7.10 (1H, s), 7.21-7.46 (10H, m), 7.69 ppm (1H, s). LC/MS (ES+): *m/z* 379.3 (M^++H). HRMS (ES): *m/z* found 379.1665; $C_{22}H_{23}N_2O_4^+$ (M^++H) requires 379.1652.

2- *N*-(4-Benzyloxy-5-methoxy-2-nitrobenzyl)-1-(2-methoxyphenyl)-methanamine **3b**

Method as for **3a** using compound **2** (5.8 g, 20 mmol), 2-methoxybenzyl amine (4.1 mL, 30 mmol) and sodium triacetoxyborohydride (6.4 g, 30 mmol) in $CHCl_3$ (100 mL) at reflux for 72 h. Flash column chromatography (PE/EtOAc 20:1→1:5) afforded compound **3b** as a yellow powder (5.9 g, 73%); mp 107-109 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 2.18 (1H, s, br), 3.80 (5H, s), 3.95 (3H, s), 4.03 (2H, s), 5.17 (2H, s), 6.83-6.92 (2H, m), 7.17 (1H, s), 7.20-7.45 (7H, m), 7.67 ppm (1H, s). HRMS (ES): *m/z* found 409.1751; $C_{23}H_{25}N_2O_5^+$ (M^++H) requires 409.1758.

N-(4-Benzyloxy-5-methoxy-2-nitrobenzyl)-1-(3-methoxyphenyl)-methanamine **3c**

Method as for **3a** using compound **2** (5.8 g, 20 mmol), 3-methoxybenzyl amine (4.1 mL, 30 mmol) and sodium triacetoxyborohydride (6.4 g, 30 mmol) in $CHCl_3$ (150 mL) at reflux for 16 h. Flash column chromatography (PE/EtOAc 20:1→1:3) afforded compound **3c** as a yellow powder (5.9 g, 72%); mp 115-116 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.93 (1H, s, br), 3.80 (3H, s), 3.81 (2H, s), 3.95 (3H, s),

4.05 (2H, s), 5.17 (2H, s), 6.77-6.81 (1H, m), 6.917-6.93 (2H, m), 7.09 (1H, s), 7.21-7.46 (6H, m), 7.68 ppm (1H, s). LC/MS (ES+): *m/z* 409.3 (M^++H). HRMS (ES): *m/z* found 409.1740; $C_{23}H_{25}N_2O_5^+$ ($M+H^+$) requires 409.1758.

N-(4-Benzyloxy-5-methoxy-2-nitrobenzyl)-1-(4-methoxyphenyl)-methanamine **3d**

Method as for **3a** using compound **2** (5.8 g, 20 mmol), 4-methoxybenzyl amine (4.1 mL, 30 mmol) and sodium triacetoxyborohydride (6.4 g, 30 mmol) in $CHCl_3$ (200 mL) at reflux for 72 h. Flash column chromatography (PE/EtOAc 20:1→1:10) afforded compound **3d** as a yellow powder (6.8 g, 84%); mp 128-129 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.87 (1H, s, br), 3.76 (2H, s), 3.79 (3H, s), 3.95 (3H, s), 4.03 (2H, s), 5.17 (2H, s), 6.86 (2H, d, *J* = 8.5 Hz), 7.10 (1H, s), 7.26 (2H, d, *J* = 8.5 Hz), 7.30-7.45 (5H, m), 7.68 ppm (1H, s). HRMS (ES): *m/z* found 409.1751; $C_{23}H_{25}N_2O_5^+$ (M^++H) requires 409.1758.

N-(4-Benzyloxy-5-methoxy-2-nitrobenzyl)-1-o-tolylmethanamine **3e**

Method as **3a** using compound **2** (5.8 g, 20 mmol), 2-methylbenzyl amine (3.6 g, 30 mmol) and sodium triacetoxyborohydride (6.4 g, 30 mmol) in $CHCl_3$ (200 mL) at reflux for 72 h. Flash column chromatography (PE/EtOAc 20:1→1:3) gave a solid that was stirred in Et_2O, filtered and dried to afford compound **3e** as a yellow powder (5.7 g, 73%); mp 92-93 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.81 (1H, s, br), 2.34 (3H, s), 3.81 (2H, s), 3.94 (3H, s), 4.11 (2H, s), 5.17 (2H, s), 7.13-7.18 (4H, m), 7.29-7.46 (6H, m), 7.68 ppm (1H, s). LC/MS (ES+): *m/z* 393.1 (M^++H). HRMS (ES): *m/z* found 393.1814; $C_{23}H_{25}N_2O_4^+$ (M^++H) requires 393.1809.

N-(4-Benzyloxy-5-methoxy-2-nitrobenzyl)-1-(2-chlorophenyl)-methanamine **3f**

Method as for **3a** using compound **2** (5.7 g, 20 mmol), 2-chlorobenzyl amine (4.25 g, 30 mmol) and sodium triacetoxyborohydride (6.4 g, 30 mmol) in $CHCl_3$ (180 mL) at reflux for 60 h. Flash column

chromatography (PE→PE/EtOAc 1:1) afforded compound **3f** as a yellow powder (5.75 g, 70%); mp 92-93 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 2.12 (1H, s, br), 3.91 (2H, s), 3.96 (3H, s), 4.06 (2H, s), 5.17 (2H, s), 7.15 (1H, s), 7.18-7.26 (2H, m), 7.28-7.46 (7H, m), 7.68 ppm (1H, s). LC/MS (ES+): *m/z* 413.3 (M^++H). HRMS (ES): *m/z* found 413.1260; $C_{22}H_{22}ClN_2O_4^+$ (M^++H) requires 413.1263.

N-(4-Benzyloxy-5-methoxy-2-nitrobenzyl)-1-(2-fluorophenyl)-methanamine **3g**

Method as for **3a** using compound **2** (5.7 g, 20 mmol), 2-fluorobenzyl amine (3.75 g, 30 mmol) and sodium triacetoxyborohydride (6.4 g, 30 mmol) in $CHCl_3$ (200 mL) at reflux for 60 h. Flash column chromatography (PE→PE/EtOAc 1:1) afforded compound **3g** as a yellow powder (5.4 g, 68%); mp 90-91 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.95 (1H, s, br), 3.86 (2H, s), 3.96 (3H, s), 4.06 (2H, s), 5.18 (2H, s), 6.99-7.13 (2H, m), 7.17 (1H, s), 7.19-7.25 (1H, m), 7.28-7.45 (6H, m), 7.68 ppm (1H, s). LC/MS (ES+): *m/z* 397.1 (M^++H). HRMS (ES): *m/z* found 397.1553; $C_{22}H_{22}FN_2O_4^+$ (M^++H) requires 397.1557.

N-(4-Benzyloxy-5-methoxy-2-nitrobenzyl)-1-(3-chlorophenyl)-methanamine **3h**

Method as **3a** using compound **2** (5.7 g, 20 mmol), 3-chlorobenzyl amine (4.25 g, 30 mmol) and sodium triacetoxyborohydride (6.4 g, 30 mmol) in $CHCl_3$ (180 mL) at reflux for 60 h. Flash column chromatography (PE→PE/EtOAc 1:1) afforded compound **3h** as a yellow powder (6.05 g, 73%); mp 90-92 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 2.05 (1H, s, br), 3.80 (2H, s), 3.95 (3H, s), 4.02 (2H, s), 5.17 (2H, s), 7.04 (1H, s), 7.21-7.25 (3H, m), 7.28-7.45 (6H, m), 7.68 ppm (1H, s). LC/MS (ES+): *m/z* 413.3 (M^++H). HRMS (ES): *m/z* found 413.1259; $C_{22}H_{22}ClN_2O_4^+$ (M^++H) requires 413.1263.

N-(4-Benzyloxy-5-methoxy-2-nitrobenzyl)-1-(2,3-dimethoxyphenyl)-methanamine **3i**

Method as for **3a** using compound **2** (5.8 g, 20 mmol), 2.3-dimethoxybenzyl amine (5 g, 30 mmol) and sodium triacetoxyborohydride (6.4 g, 30 mmol) in $CHCl_3$ (200 mL) at reflux for 48 h. Flash column chromatography (PE/EtOAc 20:1→1:10) afforded compound **3i** as a yellow powder (6.7 g, 76%); mp 105-107 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 2.07 (1H, s, br), 3.82 (5H, s), 3.85 (3H, s), 3.95 (3H, s), 4.05 (2H, s), 5.16 (2H, s), 6.83 (1H, dd, *J* = 8.0, 1.6 Hz), 6.90 (1H, dd, *J* = 7.6, 1.6 Hz), 7.01 (1H, t, *J* = 7.8 Hz), 7.19 (1H, s), 7.28-7.46 (5H, m), 7.67 ppm (1H, s). LC/MS (ES+): *m/z* 439.3 (M^++H). HRMS (ES): *m/z* found 439.1855; $C_{24}H_{27}N_2O_6^+$ (M^++H) requires 439.1864.

N-(4-Benzyloxy-5-methoxy-2-nitrobenzyl)-1-(2,5-dimethoxyphenyl)-methanamine **3j**

Method as for **3a** using compound **2** (5.8 g, 20 mmol), 2,5-dimethoxyenzyl amine (5.0 g, 30 mmol) and sodium triacetoxyborohydride (6.4 g, 30 mmol) in $CHCl_3$ (200 mL) at reflux for 24 h. Flash column chromatography (PE/EtOAc 20:1→EtOAc) afforded compound **3j** as a yellow powder (3.1 g, 35%); mp 109-110 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 2.25 (1H, s, br), 3.75 (6H, s), 3.78 (2H, s), 3.95 (3H, s), 4.03 (2H, s), 5.16 (2H, s), 6.70-6.77 (2H, m), 6.85 (1H, d, *J* = 1.9 Hz), 7.15 (1H, s), 7.30-7.45 (5H, m), 7.67 ppm (1H, s). LC/MS (ES+): *m/z* 439.3 (M^++H). HRMS (ES): *m/z* found 439.1875; $C_{24}H_{27}N_2O_6^+$ (M^++H) requires 439.1864.

N-(4-Benzyloxy-5-methoxy-2-nitrobenzyl)-1-(3,4,5-trimethoxyphenyl)-methanamine **3k**

Method as for **3a** using compound **2** (5.7 g, 20 mmol), 3,4,5-trimethoxybenzyl amine (5.9 g, 30 mmol) and sodium triacetoxyborohydride (6.35 g, 30 mmol) in $CHCl_3$ (150 mL) at reflux for 72 h. Flash column chromatography (PE/EtOAc 20:1→EtOAc) afforded compound **3k** as a yellow powder (6.6 g, 70%); mp 87-88 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 2.01 (1H, s, br), 3.77 (2H, s), 3.82 (3H, s), 3.85 (6H, s), 3.94 (3H, s), 4.04 (2H, s), 5.17 (2H, s), 6.58 (2H, s), 7.04 (1H, s), 7.29-7.45 (5H, m), 7.68 ppm (1H, s). LC/MS (ES+): *m/z* 469.2 (M^++H). HRMS (ES): *m/z* found 469.1959; $C_{25}H_{29}N_2O_7^+$ (M^++H) requires 469.1969.

2-((Benzylamino)methyl)-5-benzyloxy-4-methoxyaniline **4a**

Raney Nickel (60% slurry in water, 1 g) was washed with MeOH (3 x 2 mL). Compound **3a** (2.0 g, 5.3 mmol) in MeOH (50 mL) was added, and the mixture stirred at rt. Hydrazine hydrate (2 x 1.0 mL in 0.5 h intervals) was added dropwise, and the mixture was stirred at reflux for a total of 1 h. The reaction mixture was filtered through celite and the celite was washed with MeOH. The filtrate was concentrated in vacuo. Flash column chromatography (PE/EtOAc 3:1→EtOAc) afforded compound **4a** as a white powder (1.5 g, 82%); mp 85-86 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.46 (1H, s, br), 3.73 (2H, s), 3.77 (2H, s), 3.80 (3H, s), 4.38 (2H, s, br), 5.09 (2H, s), 6.28 (1H, s), 6.63 (1H, s), 7.22-7.43 ppm (10H, m). LC/MS (ES+): *m/z* 349.3 (M^++H). HRMS (ES): *m/z* found 349.1897; $C_{22}H_{25}N_2O_2^+$ (M^++H) requires 349.1911.

5-Benzyloxy-4-methoxy-2-((2-methoxybenzylamino)methyl)-aniline **4b**

Method as for **4a** using compound **3b** (5.3g, 13 mmol), Raney Nickel (60%, 1.5 g) and hydrazine hydrate (10 x 0.2 mL in 10 min intervals) in MeOH (200 mL) at reflux for a total of 2 h. Flash column chromatography (PE→EtOAc) afforded compound **4b** as a dark oil that slowly solidified (4.5 g, 92%); mp 60-62 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 3.69 (2H, s), 3.77 (2H, s), 3.79 (3H, s), 3.82 (3H, s), 5.09 (2H, s), 6.27 (1H, s), 6.61 (1H, s), 6.85-6.94 (2H, m), 7.17-7.44 ppm (7H, m). LC/MS (ES+): *m/z* 379.4 (M^++H). HRMS (ES): *m/z* found 379.2018; $C_{23}H_{27}N_2O_3^+$ (M^++H) requires 379.2016.

5-Benzyloxy-4-methoxy-2-((3-methoxybenzylamino)methyl)-aniline **4c**

Method as for **4a** using compound **3c** (3.5 g, 8.6 mmol), Raney Nickel (60%, 1 g) and hydrazine hydrate (10 x 0.2 mL in 10 min intervals) in MeOH (200 mL) at reflux for a total of 2 h. Flash column chromatography (PE→EtOAc) afforded compound **4c** as a dark oil that slowly solidified (2.8 g, 88%);

mp 61-63 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 3.73 (2H, s), 3.75 (2H, s), 3.79 (3H, s), 3.80 (3H, s), 5.09 (2H, s), 6.28 (1H, s), 6.64 (1H, s), 6.79 (1H, dd, *J* = 8.0, 2.5 Hz), 6.86-6.90 (2H, m), 7.21-7.44 ppm (6H, m). LC/MS (ES+): *m/z* 379.4 (M^++H). HRMS (ES): *m/z* found 379.2007; $C_{23}H_{27}N_2O_3^+$ (M^++H) requires 379.2016.

5-Benzyloxy-4-methoxy-2-((4-methoxybenzylamino)methyl)-aniline **4d**

Method as for **4a** using compound **3d** (6.7 g, 10.4 mmol), Raney Nickel (60%, 2 g) and hydrazine hydrate (10 x 0.3 mL in 10 min intervals) in MeOH (200 mL) at reflux for a total of 3 h. Flash column chromatography (PE/EtOAc 3:1→EtOAc) afforded compound **4d** as a white powder (4.5 g, 73%); mp 91-93 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 3.72 (3H, s), 3.79 (2H, s), 3.80 (2H, s), 3.81 (3H, s), 5.09 (2H, s), 6.29 (1H, s), 6.64 (1H, s), 6.86 (2H, d, *J* = 8.5 Hz), 7.22 (2H, d, *J* = 8.5 Hz), 7.25-7.44 ppm (5H, m). LC/MS (ES+): *m/z* 379.3 (M^++H). HRMS (ES): *m/z* found 379.2000; $C_{23}H_{27}N_2O_3^+$ (M^++H) requires 379.2016.

5-Benzyloxy-4-methoxy-2-((2-methylbenzylamino)methyl)aniline **4e**

Method as for **4a** using compound **3e** (5.1 g, 13 mmol), Raney Nickel (60%, 2 g) and hydrazine hydrate (10 x 0.25 mL in 10 min intervals) in MeOH (100 mL) at reflux for a total of 2 h. Flash column chromatography (PE→PE/EtOAc 1:4) afforded compound **4e** as an off-white powder (3.7 g, 79%); mp 74-75 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 2.31 (3H, s), 3.76 (2H, s), 3.78 (2H, s), 3.80 (3H, s), 4.35 (2H, s, br), 5.09 (2H, s), 6.27 (1H, s), 6.66 (1H, s), 7.16-7.18 (3H, m), 7.24-7.43 ppm (6H, m). LC/MS (ES+): *m/z* 363.3 (M^++H). HRMS (ES): *m/z* found 363.2072; $C_{23}H_{27}N_2O_2^+$ (M^++H) requires 363.2067.

5-Benzyloxy-2-((2-chlorobenzylamino)methyl)-4-methoxyaniline **4f**

Method as for **4a** using compound **3f** (5.1 g, 12.3 mmol), Raney Nickel (60%, 2 g) and hydrazine hydrate (10 x 0.25 mL in 10 min intervals) in MeOH (100 mL) at reflux for a total of 2 h. Flash column chromatography (PE→PE/EtOAc 2:3) afforded compound **4f** as a white powder (4.0 g, 85%); mp 80-81 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 3.72 (2H, s), 3.79 (3H, s), 3.86 (2H, s), 4.34 (2H, s, br), 5.09 (2H, s), 6.28 (1H, s), 6.62 (1H, s), 7.17-7.43 ppm (9H, m). LC/MS (ES+): *m/z* 383.3 ($(M^+$+H). HRMS (ES): *m/z* found 383.1509; $C_{22}H_{24}ClN_2O_2^+$ (M^++H) requires 383.1521.

5-Benzyloxy-2-((2-fluorobenzylamino)methyl)-4-methoxyaniline **4g**

Method as for **4a** using compound **3g** (4.8 g, 12.1 mmol), Raney Nickel (60%, 2 g) and hydrazine hydrate (10 x 0.25 mL in 10 min intervals) in MeOH (150 mL) at reflux for a total of 2 h. Flash column chromatography (PE→PE/EtOAc 2:3) afforded compound **4g** as an off-white powder (3.95 g, 89%); mp 87-88 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 3.72 (2H, s), 3.79 (3H, s), 3.82 (2H, s), 5.09 (2H, s), 6.27 (1H, s), 6.61 (1H, s), 6.98-7.12 (2H, m), 7.19-7.43 ppm (7H, m). LC/MS (ES+): *m/z* 367.2 (M^++H). HRMS (ES): *m/z* found 367.1814; $C_{22}H_{24}FN_2O_2$ (MH^+), 367.1816.

5-Benzyloxy-2-((3-chlorobenzylamino)methyl)-4-methoxyaniline **4h**

Method as for **4a** using compound **3h** (5 g, 12.1 mmol), Raney Nickel (60%, 2 g) and hydrazine hydrate (10 x 0.25 mL in 10 min intervals) in MeOH (150 mL) at reflux for a total of 2 h. Flash column chromatography (PE→PE/EtOAc 1:3) afforded compound **4h** as a yellow powder (3.8 g, 83%); mp 48-51 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 3.35 (1H, s, br), 3.72 (2H, s), 3.74 (2H, s), 3.79 (3H, s), 5.09 (2H, s), 6.28 (1H, s), 6.61 (1H, s), 7.15-7.43 ppm (9H, m). LC/MS (ES+): *m/z* 413.3 (M^++H).

5-Benzyloxy-2-((2,3-dimethoxybenzylamino)methyl)-4-methoxyaniline **4i**

Method as for **4a** using compound **3i** (5.5 g, 12.5 mmol), Raney Nickel (60%, 2 g) and hydrazine hydrate (10 x 0.2 mL in 10 min intervals) in MeOH (150 mL) at reflux for a total of 2 h. Flash column chromatography (PE→EtOAc) afforded compound **4i** as a white powder (3.7 g, 73%); mp 84-85 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.55 (1H, s, br), 3.69 (2H, s), 3.77 (2H, s), 3.79 (3H, s), 3.82 (3H, s), 3.86 (3H, s), 4.39 (2H, s, br), 5.09 (2H, s), 6.27 (1H, s), 6.61 (1H, s), 6.84 (2H, d, *J* = 7.7 Hz), 7.01 (1H, t, *J* = 7.7 Hz), 7.27-7.43 ppm (5H, m). LC/MS (ES+): *m/z* 409.4 (M^++H). HRMS (ES): *m/z* found 409.2130; $C_{24}H_{29}N_2O_4^+$ (M^++H) requires 409.2122.

5-Benzyloxy-2-((2,5-dimethoxybenzylamino)methyl)-4-methoxyaniline **4j**

Method as for **4a** using compound **3j** (3 g, 6.8 mmol), Raney Nickel (60%, 1 g) and hydrazine hydrate (10 x 0.2 mL in 10 min intervals) in MeOH (100 mL) at reflux for a total of 2 h. Flash column chromatography (PE→EtOAc) afforded compound **4j** as a light yellow powder (2.4 g, 86%); mp 96-98 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 3.68 (2H, s), 3.73 (2H, s), 3.75 (3H, s), 3.77 (3H, s), 3.79 (3H, s), 4.42 (2H, s, br), 5.09 (2H, s), 6.27 (1H, s), 6.61 (1H, s), 6.73-6.80 (3H, m), 7.27-7.43 ppm (5H, m). LC/MS (ES+): *m/z* 409.2 (M^++H). HRMS (ES): *m/z* found 409.2129; $C_{24}H_{29}N_2O_4^+$ (M^++H) requires 409.2122.

5-Benzyloxy-4-methoxy-2-((3,4,5-trimethoxybenzylamino)-methyl)aniline **4k**

Method as for **4a** using compound **3k** (6 g, 12.8 mmol), Raney Nickel (60%, 2 g) and hydrazine hydrate (10 x 0.3 mL in 10 min intervals) in MeOH (200 mL) at reflux for a total of 3 h. Flash column chromatography (PE/EtOAc 3:1→EtOAc) afforded compound **4k** as a yellow oil (4.9 g, 89%). ^{1}H NMR (270 MHz, $CDCl_3$): δ = 3.72 (4H, s), 3.80 (3H, s), 3.82 (3H, s), 3.84 (6H, s), 5.09 (2H, s), 6.29 (1H, s), 6.52 (2H, s), 6.64 (1H, s), 7.27-7.43 ppm (5H, m). LC/MS (ES+): *m/z* 439.3 (M^++H). HRMS (ES): *m/z* found 439.2244; $C_{25}H_{31}N_2O_5^+$ (M^++H) requires 439.2227.

3-Benzyl-7-benzyloxy-6-methoxy-3,4-dihydroquinazolin-2(1*H*)-one **5a**

Urea (0.5 g, 8.9 mmol) was stirred in DMF (0.5 mL) and heated to 180 °C in an open vessel, then compound **4a** (0.45 g, 1.24 mmol) was added and the mixture stirred at 200 °C for 2 h. The residue was cooled to rt and stirred in $CHCl_3$ (50 mL) and water (20 mL). The organic layer was separated and washed with water and brine, dried, filtered and concentrated in vacuo. Flash column chromatography (CH_2Cl_2→CH_2Cl_2/MeOH 10:1) afforded compound **5a** as a yellow powder (220 mg, 49%); mp 186-188 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 3.76 (3H, s), 4.26 (2H, s), 4.64 (2H, s), 5.06 (2H, s), 6.30 (1H, s), 6.47 (1H, s), 7.24-7.39 (10H, m), 7.65 ppm (1H, s). LC/MS (ES+): *m/z* 375.2 (M^++H). Anal. ($C_{23}H_{22}N_2O_3$) C, H, N.

7-Benzyloxy-6-methoxy-3-(2-methoxybenzyl)-3,4-dihydroquinazolin-2(1*H*)-one **5b**

Method as for **5a** using compound **4b** (0.7 g, 1.85 mmol) and urea (0.7 g, 11.7 mmol). The reaction mixture was stirred and heated to 220 °C for 1.5 h. Flash column chromatography (PE/EtOAc 5:1→1:4) gave a solid that was stirred in Et_2O, filtered and dried to afford compound **5b** as a white powder (250 mg, 33%); mp 177-178 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 3.78 (3H, s), 3.83 (3H, s), 4.32 (2H, s), 4.68 (2H, s), 5.05 (2H, s), 6.28 (1H, s), 6.48 (1H, s), 6.85-6.94 (2H, m), 7.20-7.40 (7H, m), 7.55 ppm (1H, s, br). LC/MS (ES+): *m/z* 405.2 (M^++H). HRMS (ES): *m/z* found 405.1796; $C_{24}H_{25}N_2O_4^+$ (M^++H) requires 405.1809. Anal. ($C_{24}H_{24}N_2O_4$ 0.5H_2O) C, H, N.

7-Benzyloxy-6-methoxy-3-(3-methoxybenzyl)-3,4-dihydroquinazolin-2(1*H*)-one **5c**

Method as for **5a** using compound **4c** (0.75 g, 1.97 mmol) and urea (0.8 g, 13.3 mmol) at 220 °C for 1.5 h. Flash column chromatography (PE/EtOAc 5:1→1:4) gave a solid that was stirred in Et_2O, filtered and dried to afford compound **5c** as a yellow powder (340 mg, 43%); mp 136-138 °C. ^{1}H NMR (270 MHz,

$CDCl_3$): δ = 3.76 (3H, s), 3.77 (3H, s), 4.26 (2H, s), 4.62 (2H, s), 5.06 (2H, s), 6.31 (1H, s), 6.47 (1H, s), 6.80 (1H, dd, *J* = 7.7, 2.2 Hz), 6.88 (1H, d, *J* = 2.2 Hz), 6.92 (1H, d, *J* = 7.7 Hz), 7.21-7.39 (6H, m), 7.82 ppm (1H, s). LC/MS (ES+): *m/z* 405.1 (M^++H). HRMS (ES): *m/z* found 405.1798; $C_{24}H_{25}N_2O_4^+$ (M^++H) requires 405.1809. Anal. ($C_{24}H_{24}N_2O_4$ 0.5H_2O) C, H, N.

7-Benzyloxy-6-methoxy-3-(4-methoxybenzyl)-3,4-dihydroquinazolin-2(1*H*)-one **5d**

Method as for **5a** using compound **4d** (0.7 g, 1.85 mmol) and urea (0.7 g, 11.7 mmol) at 220 °C for 1.5 h. Flash column chromatography (PE/EtOAc 5:1→1:4) gave a solid that was stirred in Et_2O, filtered and dried to afford compound **5d** as a white powder (220 mg, 30%); mp 172-174 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 3.77 (3H, s), 3.78 (3H, s), 4.23 (2H, s), 4.56 (2H, s), 5.07 (2H, s), 6.28 (1H, s), 6.47 (1H, s), 6.85 (2H, d, *J* = 8.5 Hz), 7.25-7.41 (7H, m), 7.48 ppm (1H, s, br). LC/MS (ES+): *m/z* 405.2 (M^++H). HRMS (ES): *m/z* found 405.1806; $C_{24}H_{25}N_2O_4^+$ (M^++H) requires 405.1809. Anal. ($C_{24}H_{24}N_2O_4$ 0.5H_2O) C, H, N.

7-Benzyloxy-6-methoxy-3-(2-methylbenzyl)-3,4-dihydroquinazolin-2(1*H*)-one **5e**

Method as for **5a** using compound **4e** (3.2 g, 8.8 mmol) and urea (3 g, 50 mmol) at 220 °C for 1 h. The residue was cooled to rt and stirred in $CHCl_3$ (100 mL). The organic layer was filtered and concentrated in vacuo. Flash column chromatography (CH_2Cl_2→CH_2Cl_2/EtOAc 1:1) afforded compound **5e** as an off-white powder (920 mg, 27%); mp 182-183 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 2.32 (3H, s), 3.76 (3H, s), 4.22 (2H, s), 4.67 (2H, s), 5.05 (2H, s), 6.30 (1H, s), 6.45 (1H, s), 7.12-7.39 ppm (9H, m). LC/MS (ES+): *m/z* 389.3 (M^++H). HRMS (ES): *m/z* found 389.1868; $C_{24}H_{25}N_2O_3^+$ (M^++H) requires 389.1860. Anal. ($C_{24}H_{24}N_2O_3$) C, H, N.

7-Benzyloxy-3-(2-chlorobenzyl)-6-methoxy-3,4-dihydroquinazolin-2(1*H*)-one **5f**

Method as for **5a** using compound **4f** (3.8 g, 9.9 mmol) and urea (3.6 g, 60 mmol) at 220 °C for 1 h. The resulting solid was cooled to rt and stirred in $CHCl_3$ (100 mL). The organic layer was filtered and concentrated in vacuo. Flash column chromatography (CH_2Cl_2→CH_2Cl_2/EtOAc 8:1) afforded compound **5f** as an off-white powder (850 mg, 21%); mp 190-192 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 3.76 (3H, s), 4.27 (2H, s), 4.71 (2H, s), 4.97 (2H, s), 6.33 (1H, s), 6.42 (1H, s), 7.10-7.35 (9H, m), 8.43 ppm (1H, s, br). LC/MS (ES+): *m/z* 409.2 (M^++H). HRMS (ES): *m/z* found 409.1306; $C_{23}H_{22}ClN_2O_3^+$ (M^++H) requires 409.1313. Anal. ($C_{23}H_{21}ClN_2O_3$) C, H, N.

7-Benzyloxy-3-(2-fluorobenzyl)-6-methoxy-3,4-dihydroquinazolin-2(1*H*)-one **5g**

Method as for **5a** using compound **4g** (3.6 g, 9.8 mmol) and urea (3.6 g, 60 mmol) at 220 °C for 1 h. The resulting solid was cooled to rt and stirred in $CHCl_3$ (100 mL). The organic layer was filtered and concentrated in vacuo. Flash column chromatography (CH_2Cl_2→CH_2Cl_2/EtOAc 5:1) afforded compound **5g** as an off-white powder (1.1 g, 29%); mp 209-211 °C. ^{1}H NMR (270 MHz, DMSO-d_6): δ = 3.66 (3H, s), 4.28 (2H, s), 4.58 (2H, s), 4.99 (2H, s), 6.53 (1H, s), 6.74 (1H, s), 7.15-7.24 (2H, m), 7.30-7.44 (7H, m), 9.08 ppm (1H, s, br). LC/MS (ES+): *m/z* 393.2 (M^++H). HRMS (ES): *m/z* found 393.1612; $C_{22}H_{22}FN_2O_3^+$ (M^++H) requires 393.1609. Anal. ($C_{23}H_{21}FN_2O_3$) C, H, N.

7-Benzyloxy-3-(3-chlorobenzyl)-6-methoxy-3,4-dihydroquinazolin-2(1*H*)-one **5h**

Method as for **5a** using compound **4h** (3.4 g, 8.9 mmol) and urea (3.0 g, 50 mmol) at 220 °C for 0.5 h. The resulting solid was cooled to rt and stirred in $CHCl_3$ (100 mL). The organic layer was filtered and concentrated in vacuo. Flash column chromatography (CH_2Cl_2→CH_2Cl_2/EtOAc 4:1) afforded compound **5h** as an off-white powder (1.35 g, 37%); mp 158-159 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 3.78 (3H, s), 4.27 (2H, s), 4.60 (2H, s), 5.07 (2H, s), 6.30 (1H, s), 6.48 (1H, s), 7.20-7.40 (9H, m), 7.78 ppm (1H, s, br). LC/MS (ES+): *m/z* 409.2 (M^++H). HRMS (ES): *m/z* found 409.1316; $C_{23}H_{22}ClN_2O_3^+$ (M^++H) requires 409.1313. Anal. ($C_{23}H_{21}ClN_2O_3$) C, H, N.

7-Benzyloxy-6-methoxy-3-(2,3-dimethoxybenzyl)-3,4-dihydroquinazolin-2(1*H*)-one **5i**

Method as for **5a** using compound **4i** (3.0 g, 7.3 mmol) and urea (4.5 g, 74.9 mmol) at 220 °C for 1 h. The resulting solid was cooled to rt and stirred in $CHCl_3$ (100 mL). The organic layer was filtered and concentrated in vacuo. Flash column chromatography (CH_2Cl_2→CH_2Cl_2/EtOAc 1:1) afforded compound **5i** as a white powder (780 mg, 25%); mp 166-168 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 3.76 (3H, s), 3.83 (3H, s), 3.85 (3H, s), 4.29 (2H, s), 4.71 (2H, s), 5.05 (2H, s), 6.28 (1H, s), 6.47 (1H, s), 6.82 (1H, dd, *J* = 7.7, 1.9 Hz), 6.94-7.04 (2H, m), 7.26-7.40 (5H, m), 7.56 ppm (1H, s, br). LC/MS (ES+): *m/z* 435.4 (M^++H). HRMS (ES): *m/z* found 435.1905; $C_{25}H_{27}N_2O_5^+$ (M^++H) requires 435.1914. Anal. ($C_{25}H_{26}N_2O_5$) C, H, N.

7-Benzyloxy-3-(2,5-dimethoxybenzyl)-6-methoxy-3,4-dihydroquinazolin-2(1*H*)-one **5j**

Method as for **5a** using compound **4j** (2.25 g, 5.5 mmol) and urea (2 g, 33 mmol) at 220 °C for 1 h. The resulting solid was cooled to rt and stirred in $CHCl_3$ (100 mL). The organic layer was filtered and concentrated in vacuo. Flash column chromatography (CH_2Cl_2→CH_2Cl_2/EtOAc 1:2) afforded compound **5j** as an off-white powder (630 mg, 26%); mp 160-162 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 3.68 (3H, s), 3.77 (3H, s), 3.79 (3H, s), 4.31 (2H, s), 4.66 (2H, s), 5.05 (2H, s), 6.29 (1H, s), 6.48 (1H, s), 6.73 (1H, dd, *J* = 8.8, 2.8 Hz), 6.80 (1H, d, *J* = 8.8 Hz), 6.89 (1H, d, *J* = 2.8 Hz), 7.25-7.40 (5H, m), 7.70 ppm (1H, s, br). LC/MS (ES+): *m/z* 435.3 (M^++H). HRMS (ES): *m/z* found 435.1915; $C_{25}H_{27}N_2O_5^+$ (M^++H) requires 435.1914. Anal. ($C_{25}H_{26}N_2O_5$) C, H, N.

7-Benzyloxy-6-methoxy-3-(3,4,5-trimethoxybenzyl)-3,4-dihydroquinazolin-2(1*H*)-one **5k**

Method as for **5a** using compound **4k** (0.70 g, 1.97 mmol) and urea (1.0 g, 16.6 mmol) at 220 °C for 2 h. Flash chromatography (PE/EtOAc 5:1→1:4) afforded compound **5k** as yellow powder (220 mg,

30%); mp 164-166 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 3.77 (3H, s), 3.81 (6H, s), 3.82 (3H, s), 4.25 (2H, s), 4.57 (2H, s), 5.07 (2H, s), 6.30 (1H, s), 6.49 (1H, s), 6.53 (2H, s), 7.24-7.40 (5H, m), 7.52 ppm (1H, s). LC/MS (ES+): *m/z* 465.1 (M^++H). HRMS (ES): *m/z* found 465.2002; $C_{26}H_{29}N_2O_6^+$ (M^++H) requires 465.2020. Anal. ($C_{26}H_{28}N_2O_6$ 0.5H_2O) C, H, N.

3-Benzyl-7-hydroxy-6-methoxy-3,4-dihydroquinazolin-2(1*H*)-one **6a**

Compound **5a** (250 mg, 0.67 mmol) was dissolved in EtOAc (40 mL) and MeOH (20 mL) and treated with Pd/C (10%, 30 mg) under hydrogen at rt for 16 h. The reaction mixture was filtered through celite and concentrated in vacuo. Flash column chromatography (PE/EtOAc 10:1→EtOAc) afforded compound **6a** as a cream colored powder (135 mg, 71%); mp 181-183 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 3.56 (2H, s, br), 3.81 (3H, s), 4.30 (2H, s), 4.65 (2H, s), 6.35 (1H, s), 6.47 (1H, s), 7.30-7.42 ppm (5H, m). LC/MS (ES+): *m/z* 285.0 (M^++H). HRMS (ES): *m/z* found 285.1232; $C_{16}H_{17}N_2O_3^+$ (M^++H) requires 285.1234. Anal. ($C_{16}H_{16}N_2O_3$ 0.25H_2O) C, H, N.

7-Hydroxy-6-methoxy-3-(2-methoxybenzyl)-3,4-dihydroquinazolin-2(1*H*)-one **6b**

Method as **6a** using compound **5b** (170 mg, 0.42 mmol) and Pd/C (10%, 20 mg) in THF (10 mL) and MeOH (20 mL) under hydrogen at rt for 18 h. The residue was stirred in Et_2O/EtOAc (10:1), filtered and dried to afford compound **6b** as a white powder (120 mg, 91%); mp 183-185 °C. ^{1}H NMR (270 MHz, $CDCl_3$/CD_3OD 5:1): δ = 3.26 (2H, s, br), 3.70 (3H, s), 3.77 (3H, s), 4.24 (2H, s), 4.57 (2H, s), 6.24 (1H, s), 6.37 (1H, s), 6.78-6.89 (2H, m), 7.13-7.22 ppm (2H, m). LC/MS (ES+): *m/z* 315.1 (M^++H). HRMS (ES): *m/z* found 315.1340; $C_{17}H_{19}N_2O_4^+$ (M^++H) requires 315.1339. Anal. ($C_{17}H_{18}N_2O_4$ 0.25H_2O) C, H, N.

7-Hydroxy-6-methoxy-3-(3-methoxybenzyl)-3,4-dihydroquinazolin-2(1*H*)-one **6c**

Method as for **6a** using compound **5c** (230 mg, 0.57 mmol) and Pd/C (10%, 30 mg) in THF (10 mL) and MeOH (10 mL) under hydrogen at rt for 18 h. The residue was stirred in Et_2O/EtOAc (1:1), filtered and dried to afford compound **6c** as an off-white powder (155 mg, 87%); mp 197-199 °C. ^{1}H NMR (270 MHz, $CDCl_3$/CD_3OD 5:1): δ = 3.48 (2H, s), 3.68 (3H, s), 3.71 (3H, s), 4.18 (2H, s), 4.50 (2H, s), 6.24 (1H, s), 6.35 (1H, s), 6.72-6.83 (3H, m), 7.17 ppm (1H, t, *J* = 7.7 Hz). LC/MS (ES+): *m/z* 315.1 (M^++H). HRMS (ES): *m/z* found 315.1340; $C_{17}H_{19}N_2O_4^+$ (M^++H) requires 315.1339. Anal. ($C_{17}H_{18}N_2O_4$ 0.25H_2O) C, H, N.

7-Hydroxy-6-methoxy-3-(4-methoxybenzyl)-3,4-dihydroquinazolin-2(1*H*)-one **6d**

Method as for **6a** using compound **5d** (180 mg, 0.44 mmol) and Pd/C (10%, 30mg) in THF (10 mL) and MeOH (20 mL) under hydrogen at rt for 18 h. The residue was stirred in Et_2O/EtOAc (10:1), filtered and dried to afford compound **6d** as a white powder (110 mg, 79%); mp 175-177 °C. ^{1}H NMR (270 MHz, $CDCl_3$/CD_3OD 5:1): δ = 2.42 (2H, s, br), 3.73 (3H, s), 3.75 (3H, s), 4.18 (2H, s), 4.51 (2H, s), 6.28 (1H, s), 6.39 (1H, s), 6.83 (2H, d, *J* = 8.8 Hz), 7.21 ppm (2H, d, *J* = 8.8 Hz). LC/MS (ES+): *m/z* 315.0 (M^++H). HRMS (ES): *m/z* found 315.1336; $C_{17}H_{19}N_2O_4^+$ (M^++H) requires 315.1339. Anal. ($C_{17}H_{18}N_2O_4$ 0.25H_2O) C, H, N.

7-Hydroxy-6-methoxy-3-(2-methylbenzyl)-3,4-dihydroquinazolin-2(1*H*)-one **6e**

Method as for **6a** using compound **5e** (525 mg, 1.35 mmol) and Pd/C (10%, 60 mg) in MeOH (120 mL) under hydrogen at rt for 18 h. The residue was stirred in EtOAc (10 mL), filtered and dried to afford compound **6e** as a white powder (240 mg, 60%); mp 205-207 °C. ^{1}H NMR (270 MHz, DMSO-d_6): δ = 2.36 (3H, s), 3.62 (3H, s), 4.16 (2H, s), 4.52 (2H, s), 6.35 (1H, s), 6.60 (1H, s), 7.12-7.18 (4H, m), 9.00 ppm (2H, s, br). LC/MS (ES+): *m/z* 299.1 (M^++H). HRMS (ES): *m/z* found 299.1384; $C_{17}H_{19}N_2O_3^+$ (M^++H) requires 299.1390. Anal. ($C_{17}H_{18}N_2O_3$ 1.5H_2O) C, H, N.

3-(2-Chlorobenzyl)-7-hydroxy-6-methoxy-3,4-dihydroquinazolin-2(1*H*)-one **6f**

Compound **5f** (350 mg, 0.86 mmol) and methanesulfonic acid (2.5 mL) were stirred in CH_2Cl_2 (2.5 mL) at rt for 24 h. $NaHCO_3$ (sat.) was added, and the mixture was extracted with EtOAc. The organic layer was washed with water, brine and dried ($MgSO_4$), filtered and concentrated in vacuo. Flash column chromatography (PE→EtOAc) afforded compound **6f** as a white powder (100 mg, 37%); mp 208-210 °C. ^{1}H NMR (270 MHz, DMSO-d_6): δ = 3.65 (3H, s), 4.28 (2H, s), 4.61 (2H, s), 6.34 (1H, s), 6.64 (1H, s), 7.26-7.33 (3H, m), 7.47 (1H, dd, *J* = 8.8, 2.2 Hz), 9.01 (1H, s), 9.08 ppm (1H, s). LC/MS (ES+): *m/z* 319.1 (M^++H). HRMS (ES): *m/z* found 319.0838; $C_{16}H_{16}ClN_2O_3^+$ (M^++H) requires 319.0844. Anal. ($C_{16}H_{15}ClN_2O_3$) C, H, N.

3-(2-Fluorobenzyl)-7-hydroxy-6-methoxy-3,4-dihydroquinazolin-2(1*H*)-one **6g**

Method as for **6a** using compound **5g** (510 mg, 1.26 mmol) and Pd/C (10%, 70 mg) in MeOH (100 mL) under hydrogen at rt for 4 h. The residue was crystallized from EtOAc, filtered and dried to afford compound **6g** as a white powder (360 mg, 92%); mp 196-198 °C. ^{1}H NMR (270 MHz, DMSO-d_6): δ = 3.64 (3H, s), 4.24 (2H, s), 4.57 (2H, s), 6.32 (1H, s), 6.64 (1H, s), 7.16-7.24 (2H, m), 7.29-7.37 (2H, m), 9.00 (1H, s, br), 9.04 ppm (1H, s, br). LC/MS (ES+): *m/z* 303.1 (M^++H). HRMS (ES): *m/z* found 303.1138; $C_{16}H_{16}FN_2O_3^+$ (M^++H) requires 303.1139. Anal. ($C_{16}H_{15}FN_2O_3$) C, H, N.

3-(3-Chlorobenzyl)-7-hydroxy-6-methoxy-3,4-dihydroquinazolin-2(1*H*)-one **6h**

Method as for **6a** using **5h** (310 mg, 0.76 mmol) and Pd/C (10%, 40 mg) in THF (20 mL) in MeOH (20 mL) under hydrogen at rt for 0.75 h. Flash column chromatography (PE→PE/EtOAc 1:10) afforded compound **6h** as a white powder (145 mg, 60%); mp 197-199 °C. ^{1}H NMR (270 MHz, DMSO-d_6): δ = 3.64 (3H, s), 4.21 (2H, s), 4.51 (2H, s), 6.33 (1H, s), 6.63 (1H, s), 7.25-7.40 (4H, m), 9.01 (1H, s), 9.05

ppm (1H, s). LC/MS (ES+): *m/z* 319.0 (M^++H). HRMS (ES): *m/z* found 319.0842; $C_{16}H_{16}ClN_2O_3^+$ (M^++H) requires 319.0844. Anal. ($C_{16}H_{15}ClN_2O_3$) C, H, N.

3-(2,3-Dimethoxybenzyl)-7-hydroxy-6-methoxy-3,4-dihydroquinazolin-2(1*H*)-one **6i**

Method as for **6a** using compound **5i** (525 mg, 1.21 mmol) and Pd/C (10%, 60 mg) in MeOH (40 mL) under hydrogen at 65 °C for 0.5 h. The residue was stirred in EtOAc (20 mL), filtered and dried to afford compound **6i** as an off-white powder (230 mg, 57%); mp 210-213 °C. ^{1}H NMR (270 MHz, DMSO-d_6): δ = 3.63 (3H, s), 3.72 (3H, s), 3.79 (3H, s), 4.19 (2H, s), 4.52 (2H, s), 6.31 (1H, s), 6.61 (1H, s), 6.76 (1H, d, *J* = 6.9 Hz), 6.94-7.05 (2H, m), 8.98 ppm (2H, s, br). LC/MS (ES+): *m/z* 345.2 (M^++H). HRMS (ES): *m/z* found 345.1435; $C_{18}H_{21}N_2O_5^+$ (M^++H) requires 345.1445. Anal. ($C_{18}H_{20}N_2O_5$) C, H, N.

3-(2,5-Dimethoxybenzyl)-7-hydroxy-6-methoxy-3,4-dihydroquinazolin-2(1*H*)-one **6j**

Method as for **6a** using compound **5j** (390 mg, 0.9 mmol) and Pd/C (10%, 50 mg) in MeOH (120 mL) under hydrogen at rt for 18 h. The residue was crystallized from EtOAc, filtered and dried to afford compound **6j** as a white powder (220 mg, 71%); mp 188-189 °C. ^{1}H NMR (270 MHz, DMSO-d_6): δ = 3.63 (3H, s), 3.64 (3H, s), 3.75 (3H, s), 4.22 (2H, s), 4.45 (2H, s), 6.32 (1H, s), 6.63 (1H, s), 6.67 (1H, d, *J* = 3.0 Hz), 6.80 (1H, dd, *J* = 8.6, 3.0 Hz), 6.93 (1H, d, *J* = 8.6 Hz), 8.97 ppm (2H, s, br). LC/MS (ES+): *m/z* 345.2 (M^++H). HRMS (ES): *m/z* found 345.1442; $C_{18}H_{21}N_2O_5^+$ (M^++H) requires 345.1445. Anal. ($C_{18}H_{20}N_2O_5$) C, H, N.

7-Hydroxy-6-methoxy-3-(3,4,5-trimethoxybenzyl)-3,4-dihydroquinazolin-2(1*H*)-one **6k**

Method as for **6a** using compound **5k** (195 mg, 0.42 mmol) and Pd/C (10%, 20 mg) in MeOH (30 mL) under hydrogen at rt for 16 h. The residue was stirred in Et_2O/EtOAc (1:1), filtered and dried to afford

compound **6k** as a white powder (145 mg, 71%), mp 174-176 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 3.78 (3H, s), 3.82 (9H, s), 4.24 (2H, s), 4.57 (2H, s), 5.79 (1H, s), 6.37 (1H, s), 6.45 (1H, s), 6.54 (2H, s), 7.60 ppm (1H, s). LC/MS (ES+): *m/z* 375.2 (M^++H). HRMS (ES): *m/z* found 375.1539; $C_{19}H_{23}N_2O_6^+$ (M^++H) requires 375.1551. Anal. ($C_{19}H_{22}N_2O_6$ 0.5H_2O) C, H, N.

3-Benzyl-6-methoxy-7-sulfamoyloxy-3,4-dihydroquinazolin-2(1*H*)-one **7a**

Compound **6a** (80 mg, 0.28 mmol) and sulfamoyl chloride (0.57 M, 1.5 mL, 0.84 mmol) were reacted in anhydrous DMA (1.0 mL) at rt for 18 h. Water (10 mL) was added, and the mixture was extracted with EtOAc (80 mL). The organic layer was separated, washed with water and brine, dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was stirred in Et_2O, filtered and dried to afford compound **7a** as a light yellow powder (50 mg, 49%); mp 118-120 °C. ^{1}H NMR (270 MHz, $CDCl_3$/CD_3OD 1:1): δ = 3.73 (3H, s), 4.25 (2H, s), 4.54 (2H, s), 6.57 (1H, s) 6.77 (1H, s), 7.17-7.30 ppm (5H, m). LC/MS (ES+): *m/z* 364.0 (M^++H). HRMS (ES): *m/z* found 364.0958; $C_{16}H_{18}N_3O_5S^+$ (M^++H) requires 364.0962. Anal. ($C_{16}H_{17}N_3O_5S$ 0.5H_2O) C, H, N.

6-Methoxy-3-(2-methoxybenzyl)-7-sulfamoyloxy-3,4-dihydroquinazolin-2(1*H*)-one **7b**

Method as for **7a** using compound **6b** (90 mg, 0.29 mmol) and sulfamoyl chloride (1.2 mmol) in DMA (1.0 mL) at rt for 18 h. The residue was stirred in Et_2O, filtered and dried to afford compound **7b** as a white powder (70 mg, 71%); mp 160-161 °C. ^{1}H NMR (270 MHz, DMSO-d_6): δ = 3.71 (3H, s), 3.83 (3H, s), 4.35 (2H, s), 4.52 (2H, s), 6.86 (1H, s), 6.90-6.96 (2H, m), 7.03 (1H, d, *J* = 8.0 Hz), 7.14 (1H, dd, *J* = 7.2, 2.0 Hz), 7.24-7.30 (1H, m), 7.92 (2H, s), 9.23 ppm (1H, s). LC/MS (ES+): *m/z* 394.2 (M^++H). HRMS (ES): *m/z* found 394.1070; $C_{17}H_{20}N_3O_6S^+$ (M^++H) requires 394.1067. Anal. ($C_{17}H_{19}N_3O_6S$ 0.5H_2O) C, H, N.

6-Methoxy-3-(3-methoxybenzyl)-7-sulfamoyloxy-3,4-dihydroquinazolin-2(1*H*)-one **7c**

Method as for **7a** using compound **6c** (101 mg, 0.32 mmol) and sulfamoyl chloride (1.0 mmol) in DMA (1.0 mL) at rt for 18 h. The residue was stirred in Et_2O, filtered and dried to afford compound **7c** as a white powder (95 mg, 76%); mp 185-187 °C. ^{1}H NMR (270 MHz, DMSO-d_6): δ = 3.70 (3H, s), 3.74 (3H, s), 4.30 (2H, s), 4.52 (2H, s), 6.82-6.90 (4H, m), 6.93 (1H, s), 7.28 (1H, t, *J* = 7.7 Hz), 7.93 (2H, s), 9.28 ppm (1H, s). LC/MS (ES+): *m/z* 394.1 (M^++H). HRMS (ES): *m/z* found 394.1063; $C_{17}H_{20}N_3O_6S^+$ (M^++H) requires 394.1067. Anal. ($C_{17}H_{19}N_3O_6S$ 0.5H_2O) C, H, N.

6-Methoxy-3-(4-methoxybenzyl)-7-sulfamoyloxy-3,4-dihydroquinazolin-2(1*H*)-one **7d**

Method as for **7a** using compound **6d** (80 mg, 0.25 mmol) and sulfamoyl chloride (1.0 mmol) in DMA (1.0 mL) at rt for 18 h. The residue was stirred in Et_2O, filtered and dried to afford compound **7d** as a white powder (70 mg, 71%); mp 123-125 °C. ^{1}H NMR (270 MHz, DMSO-d_6): δ = 3.68 (3H, s), 3.73 (3H, s), 4.25 (2H, s), 4.46 (2H, s), 6.83 (1H, s), 6.90 (1H, s), 6.92 (2H, d, *J* = 7.7 Hz), 7.24 (2H, d, *J* = 8.8 Hz), 7.91 (2H, s), 9.23 ppm (1H, s). LC/MS (ES+): *m/z* 394.2 (M^++H). HRMS (ES): *m/z* found 394.1049; $C_{17}H_{20}N_3O_6S^+$ (M^++H) requires 394.1067. Anal. ($C_{17}H_{19}N_3O_6S$ 0.5H_2O) C, H, N.

6-Methoxy-3-(2-methylbenzyl)-7-sulfamoyloxy-3,4-dihydroquinazolin-2(1*H*)-one **7e**

Method as for **7a** using compound **6e** (120 mg, 0.4 mmol) and sulfamoyl chloride (1.6 mmol) in DMA (1.5 mL) at rt for 18 h. The residue was stirred in Et_2O/EtOAc (1:1), filtered and dried to afford compound **7e** as a white powder (110 mg, 73%); mp 181-183 °C. ^{1}H NMR (270 MHz, DMSO-d_6): δ = 2.27 (3H, s), 3.68 (3H, s), 4.27 (2H, s), 4.55 (2H, s), 6.84 (1H, s), 6.90 (1H, s), 7.18 (4H, s), 7.91 (2H, s), 9.26 ppm (1H, s). LC/MS (ES+): *m/z* 378.2 (M^++H). HRMS (ES): *m/z* found 378.1116; $C_{17}H_{20}N_3O_5S^+$ (M^++H) requires 378.1118. Anal. ($C_{17}H_{19}N_3O_5S$) C, H, N.

3-(2-Chlorobenzyl)-6-methoxy-7-sulfamoyloxy-3,4-dihydroquinazolin-2(1*H*)-one **7f**

Method as for **7a** using compound **6f** (95 mg, 0.3 mmol) and sulfamoyl chloride (1.2 mmol) in DMA (1.0 mL) at rt for 18 h. Flash column chromatography (PE→PE/EtOAc 1:4) afforded compound **7f** as a white powder (45 mg, 38%); mp 136-138 °C. ^{1}H NMR (270 MHz, DMSO-d_6): δ = 3.69 (3H, s), 4.38 (2H, s), 4.63 (2H, s), 6.86 (1H, s), 6.93 (1H, s), 7.29-7.36 (3H, m), 7.46-7.50 (1H, m), 7.95 (2H, s), 9.33 ppm (1H, s). LC/MS (ES+): *m/z* 398.1 (M^++H). HRMS (ES): *m/z* found 398.0579; $C_{16}H_{17}ClN_3O_5S^+$ (M^++H) requires 399.0572. Anal. ($C_{16}H_{16}ClN_3O_5S$) C, H, N.

3-(2-Fluorobenzyl)-6-methoxy-7-sulfamoyloxy-3,4-dihydroquinazolin-2(1*H*)-one **7g**

Method as for **7a** using compound **6g** (180 mg, 0.6 mmol) and sulfamoyl chloride (2.4 mmol) in DMA (1.5 mL) at rt for 18 h. The residue was stirred in Et_2O/EtOAc (1:1), filtered and dried to afford compound **7g** as a white powder (175 mg, 76%); mp 143-145 °C. ^{1}H NMR (270 MHz, DMSO-d_6): δ = 3.69 (3H, s), 4.35 (2H, s), 4.60 (2H, s), 6.84 (1H, s), 6.94 (1H, s), 7.17-7.25 (2H, m), 7.31-7.39 (2H, m), 7.91 (2H, s), 9.28 ppm (1H, s). LC/MS (ES+): *m/z* 382.2 (M^++H). HRMS (ES): *m/z* found 382.0859; $C_{16}H_{17}FN_3O_5S^+$ (M^++H) requires 382.0867. Anal. ($C_{16}H_{16}FN_3O_5S$) C, H, N.

3-(3-Chlorobenzyl)-6-methoxy-7-sulfamoyloxy-3,4-dihydroquinazolin-2(1*H*)-one **7h**

Method as for **7a** using compound **6h** (100 mg, 0.31 mmol) and sulfamoyl chloride (1.2 mmol) in DMA (1.0 mL) at rt for 18 h. The residue was stirred in Et_2O, filtered and dried to afford compound **7h** as a white powder (95 mg, 77%); mp 143-145 °C. ^{1}H NMR (270 MHz, DMSO-d_6): δ = 3.69 (3H, s), 4.31 (2H, s), 4.54 (2H, s), 6.85 (1H, s), 6.92 (1H, s), 7.26-7.42 (4H, m), 7.91 (2H, s), 9.30 ppm (1H, s). LC/MS (ES+): *m/z* 398.2 (M^++H). HRMS (ES): *m/z* found 398.0567; $C_{16}H_{17}ClN_3O_5S^+$ (M^++H) requires 399.0572. Anal. ($C_{16}H_{16}ClN_3O_5S$) C, H, N.

3-(2,3-Dimethoxybenzyl)-7-sulfamoyloxy-6-methoxy-3,4-dihydroquinazolin-2(1*H*)-one **7i**

Method as for **7a** using compound **6i** (117 mg, 0.35 mmol) and sulfamoyl chloride (1.4 mmol) in DMA (1.0 mL) at rt for 18 h. The residue was stirred in Et_2O/EtOAc (1:1), filtered and dried to afford compound **7i** as a white powder (95 mg, 64%); mp 164-166 °C. ^{1}H NMR (270 MHz, DMSO-d_6): δ = 3.68 (3H, s), 3.74 (3H, s), 3.80 (3H, s), 4.30 (2H, s), 4.55 (2H, s), 6.78 (1H, d, *J* = 7.2 Hz), 6.83 (1H, s), 6.92 (1H, s), 6.95-7.04 (2H, m), 7.91 (2H, s), 9.23 ppm (1H, s). LC/MS (ES+): *m/z* 424.0 (M^++H). HRMS (ES): *m/z* found 424.1165; $C_{18}H_{22}N_3O_7S^+$ (M^++H) requires 424.1173. Anal. ($C_{18}H_{21}N_3O_7S$) C, H, N.

3-(2,5-Dimethoxybenzyl)-7-sulfamoyloxy-6-methoxy-3,4-dihydroquinazolin-2(1*H*)-one **7j**

Method as for **7a** using compound **6j** (100 mg, 0.29 mmol) and sulfamoyl chloride (1.4 mmol) in DMA (1.0 mL) at rt for 18 h. The residue was stirred in Et_2O/EtOAc (1:1), filtered and dried to afford compound **7j** as a white powder (95 mg, 77%); mp 176-178 °C. ^{1}H NMR (270 MHz, DMSO-d_6): δ = 3.65 (3H, s), 3.70 (3H, s), 3.77 (3H, s), 4.33 (2H, s), 4.47 (2H, s), 6.68 (1H, d, *J* = 3.0 Hz), 6.82 (1H, dd, *J* = 8.8, 3.0 Hz), 6.84 (1H, s), 6.93 (1H, s), 6.95 (1H, d, *J* = 8.8 Hz), 7.91 (2H, s), 9.23 ppm (1H, s). LC/MS (ES+): *m/z* 424.1 (M^++H). HRMS (ES): *m/z* found 424.1178; $C_{18}H_{22}N_3O_7S^+$ (M^++H) requires 424.1173. Anal. ($C_{18}H_{21}N_3O_7S$) C, H, N.

6-Methoxy-7-sulfamoyloxy-3-(3,4,5-trimethoxybenzyl)-3,4-dihydroquinazolin-2(1*H*)-one **7k**

Method as for **7a** using compound **6k** (101 mg, 0.27 mmol) and sulfamoyl chloride (1.0 mmol) in DMA (1.0 mL) at rt for 18 h. The residue was stirred in Et_2O, filtered and dried to afford compound **7k** as a cream colored solid (95 mg, 78%); mp 112-114 °C. ^{1}H NMR (270 MHz, DMSO-d_6): δ = 3.63 (3H, s), 3.69 (3H, s), 3.74 (6H, s), 4.28 (2H, s), 4.47 (2H, s), 6.61 (2H, s), 6.84 (1H, s), 6.93 (1H, s), 7.92 (2H,

s), 9.25 ppm (1H, s). LC/MS (ES+): *m/z* 454.2 (M^++H). HRMS (ES): *m/z* found 454.1279; $C_{19}H_{24}N_2O_8S^+$ (M^++H) requires 454.1279. Anal. ($C_{19}H_{23}N_3O_8S$) C, H, N.

Associated Content

Supplementary Information: The Supplementary Information is available free of charge on the ACS Publication website at http://pubs.ac.org. ^{13}C NMR Data and elemental microanalyses for selected compounds, full structures of all dihydroquinazolinones, tetrahydroisoquinolines and derivatives evaluated in the STS assay, data collection and refinement statistics for X-ray crystal structure of T_2R-TTL-**7j** complex, inhibition of steroid sulfatase at 10 μM inhibitor concentration, docking of **7b** and **7f** into the crystal structure of human STS and *in vivo* data of **7b** (PDF). SMILES molecular formula strings and some data (CSV).

Accession Codes: The PDB access code for the structure of **7j** bound to the tubulin dimer is 5OSK. Authors will release the atomic coordinates and experimental data upon article publication.

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Disclaimer: The content of this paper is solely the responsibility of the authors and does not necessarily reflect the official views of the National Institutes of Health.

Abbreviations Used: DHQ, dihydroquinazolinone; THIQ, tetrahydroisoquinoline; STS, steroid sulfatase; FGly, formylglycine; CAIX, carbonic anhydrase IX.

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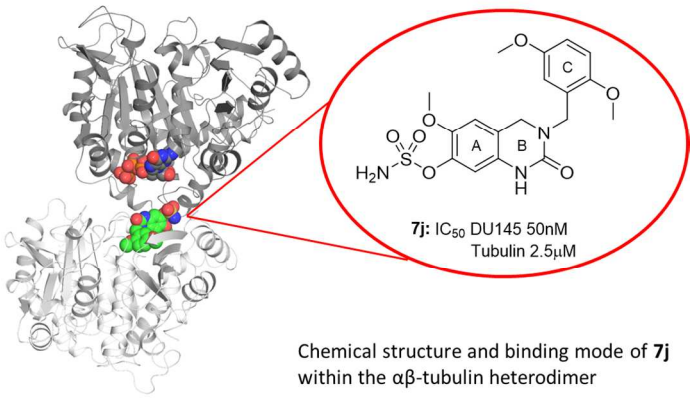
Table of Contents Graphic



Chemical structure and binding mode of **7j** within the αβ-tubulin heterodimer