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. These agents were developed as part of a programme addressing design of inhibitors of the emerging drug target steroid sulfatase (STS). 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. 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, 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, 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. 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.

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. 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)\textsuperscript{14} (Scheme 1).

**Scheme 1\textsuperscript{a}** Synthesis of quinazolinone-based microtubule disruptors.

![Scheme 1](image)

\textsuperscript{a}Reagents and conditions: i) ArCH\textsubscript{2}NH\textsubscript{2}, NaB(OAc)\textsubscript{3}H, CHCl\textsubscript{3}, reflux; ii) Raney Ni, N\textsubscript{2}H\textsubscript{4}·H\textsubscript{2}O, MeOH, reflux; iii) Urea, 220 °C; iv) H\textsubscript{2}, Pd/C, THF/MeOH, rt; v) CH\textsubscript{3}SO\textsubscript{3}H, CH\textsubscript{2}Cl\textsubscript{2}, rt; vi) H\textsubscript{2}NSO\textsubscript{2}Cl, 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\textsubscript{50} 33 μM), the 2'-methoxy derivative proved extremely interesting, with the 6-hydroxy compound and its 6-\textit{O}-sulfamate,
respectively, exhibiting micromolar and nanomolar GI\textsubscript{50}'s. This finding, that sulfamate 7\textsubscript{b} was more active than 6\textsubscript{b}, 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\textsubscript{50}'s of 0.3 µM and 2.2 µM for 7\textsubscript{b} and 6\textsubscript{b} respectively). These preliminary results indicated that 2'-substitution was worthy of further investigation, and a second set of phenols 6\textsubscript{e-k} and their sulfamates 7\textsubscript{e-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 (7\textsubscript{g} GI\textsubscript{50} 4.8 µM). In strong contrast, the 2'-chloro derivative 7\textsubscript{f}, which offers unhindered H-bond acceptor potential, showed excellent activity (GI\textsubscript{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 7\textsubscript{h} proved significantly more active (ca 10-fold) than the corresponding 3'-methoxy compound 7\textsubscript{c}, 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 \textit{in vitro}.\textsuperscript{a}

\begin{tabular}{lcccccc}
\hline
compd & R\textsuperscript{1} & R\textsuperscript{2} & R\textsuperscript{3} & R\textsuperscript{4} & R\textsuperscript{5} & GI\textsubscript{50} (µM) \\
DU-145 & MDA MB-231 \\
\hline
6\textsubscript{a} & H & H & H & H & H & >100 & >100 \\
7\textsubscript{a} & SO\textsubscript{2}NH\textsubscript{2} & H & H & H & H & >100 & >100 \\
\hline
\end{tabular}
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<sub>50</sub> 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<sub>50</sub> 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. 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.$^a$

<table>
<thead>
<tr>
<th>compd</th>
<th>Tubulin assembly IC$_{50}$ (µM)</th>
<th>Colchicine binding (% Inhibition)</th>
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<tr>
<td>CA-4</td>
<td>1.1 ± 0.1</td>
<td>99 ± 0.6</td>
</tr>
<tr>
<td>7b</td>
<td>2.5 ± 0.4</td>
<td>55 ± 2</td>
</tr>
<tr>
<td>7f</td>
<td>5.0 ± 0.7</td>
<td>43 ± 0.09</td>
</tr>
<tr>
<td>7i</td>
<td>8.6 ± 0.4</td>
<td>ND$^b$</td>
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Table 1:

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<tbody>
<tr>
<td>7j</td>
<td>2.5 ± 0.1</td>
<td>61 ± 2</td>
</tr>
<tr>
<td>7k</td>
<td>5.6 ± 0.02</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Values are the mean ±SD of at least two determinations. 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 T2R-TTL crystal\(^1\) and solved the T2R-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\(^2\) 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)
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-sulphonamide 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.

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 T2R-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.\textsuperscript{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.\textsuperscript{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\textsubscript{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 T2R-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,\textsuperscript{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,\textsuperscript{7} as for the well-established aromatase inhibition. As a comparison, some previously synthesized tetrahydroisoquinoline derivatives 8-14\textsuperscript{9, 11-12} and the well-known potent inhibitor irosustat 15\textsuperscript{7, 20} (all structures see S5 in SI) were also evaluated alongside the quinazolinone derivatives, with the expectation that, like most aryl sulfamate esters,\textsuperscript{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 Ca²⁺ 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.
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. 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. 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,\textsuperscript{21} and this is an emerging area of clinical interest.\textsuperscript{22}

On the basis of its promising \textit{in vitro} activity, 7b was selected as an early lead for a preliminary evaluation in an RPMI 8226 \textit{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).\textsuperscript{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 \textit{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 \textit{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.\textsuperscript{7, 23} Sulfamoylation provides attractive pharmaceutical and pharmacodynamic advantages, as well as conferring oral activity,\textsuperscript{24} something much less common in antimitotic drugs. For example, one agent of promise is the steroid \textit{bis}-sulfamate 1a (STX140).\textsuperscript{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 STX14012 in xenograft models in vivo26 and more widely27 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% CO₂ 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 (GI₅₀). All experiments were performed in triplicate.

Tubulin Assays. Bovine brain tubulin, prepared as described previously, was used in the studies presented here. Assembly IC₅₀’s were determined as described in detail elsewhere. 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 [3H]colchicine to tubulin was described in detail previously.30 Reaction mixtures contained 0.1 mg/mL (1.0 µM) tubulin, 5.0 µM [3H]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 T2R-TTL were grown as previously described16, 32 and soaked for 3 h at 20 °C in the reservoir solution (10% PEG 4k, 16% glycerol, 30 mM MgCl2, 30 mM CaCl2, 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 cryosolution (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 T2R-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 T2R-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. 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 [6,7-3H] E1S (4 × 10^5 dpm, Perkin-ElmerLS, Boston, MA, USA) adjusted to a final concentration of 20 µM with unlabeled E1S. [4-14C] 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 3H and 14C 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) 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. CHCl₃, CH₂Cl₂, N,N-dimethylacetamide (DMA), N,N-dimethylformamide (DMF) and tetrahydrofuran (THF) were purchased from Aldrich and stored under a positive pressure of N₂ after use. Sulfamoyl chloride was prepared by an adaptation of the method of Appel and Berger and was stored in the refrigerator under positive pressure of N₂ as a solution in toluene as described by Woo et al. An appropriate volume of this solution was freshly concentrated in vacuo immediately before use. Compound 2 was prepared according to literature procedure. Reactions were carried out at room temperature unless stated otherwise. Flash column chromatography was performed on silica gel (MatrexC60). ¹H NMR and ¹³C 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 (¹³C 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 CH₃CN/H₂O or MeOH/H₂O (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$_3$): $\delta$ = 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$_2$O$_4$ (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$_3$): $\delta$ = 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). LC/MS (ES+): $m/z$ 409.1751; C$_{23}$H$_{25}$N$_2$O$_5$ (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$_3$): $\delta$ = 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), 6.91-6.93 (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_{2}O_{5}^+ (M+H^+) requires 409.1758.

N-(4-Benzzyloxy-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. ^1H 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_{2}O_{5}^+ (M+H^+) requires 409.1758.

N-(4-Benzzyloxy-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. ^1H 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_{2}O_{4}^+ (M+H^+) requires 393.1809.

N-(4-Benzzyloxy-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\(_3\)): \(\delta = 2.12\) (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\(_2\)O\(_4\)+ (M\(^{+}\)+H) requires 413.1263.

\(N\)-(4-Benzylxy-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\(_3\)): \(\delta = 1.95\) (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\(_2\)O\(_4\)+ (M\(^{+}\)+H) requires 397.1557.

\(N\)-(4-Benzylxy-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\(_3\)): \(\delta = 2.05\) (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\(_2\)O\(_4\)+ (M\(^{+}\)+H) requires 413.1263.

\(N\)-(4-Benzylxy-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 CHCl₃ (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. ¹H NMR (270 MHz, CDCl₃): 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; C₂₄H₂₇N₂O₆⁺ (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 CHCl₃ (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. ¹H NMR (270 MHz, CDCl₃): 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; C₂₄H₂₇N₂O₆⁺ (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 CHCl₃ (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. ¹H NMR (270 MHz, CDCl₃): δ = 2.01 (1H, s, br), 3.77 (6H, s), 3.78 (2H, s), 3.82 (2H, 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; C₂₅H₂₉N₂O₇⁺ (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\(_3\)): \(\delta = 1.46\) (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\(_2\)O\(_2\) (M\(^+\)+H) requires 349.1911.

5-Benzyl-oxo-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\(_3\)): \(\delta = 3.69\) (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 ppm (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\(_2\)O\(_3\) (M\(^+\)+H) requires 379.2016.

5-Benzyl-oxo-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.  

\[ ^1 \text{H NMR (270 MHz, CDCl}_3\]: } \delta = 3.73 \text{ (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 \text{ Hz), 6.86-6.90 (2H, m), 7.21-7.44 \text{ ppm (6H, m). LC/MS (ES+): } m/z \text{ 379.4 (M}^+\text{+H). HRMS (ES): m/z found 379.2007; C}_{23}\text{H}_{27}\text{N}_2\text{O}_3^+ (M}^+\text{+H) requires 379.2016.}

5-Benzylxoy-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.  

\[ ^1 \text{H NMR (270 MHz, CDCl}_3\]: } \delta = 3.72 \text{ (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 \text{ Hz), 7.22 (2H, d, } J = 8.5 \text{ Hz), 7.25-7.44 \text{ ppm (5H, m). LC/MS (ES+): } m/z \text{ 379.3 (M}^+\text{+H). HRMS (ES): m/z found 379.2000; C}_{23}\text{H}_{27}\text{N}_2\text{O}_3^+ (M}^+\text{+H) requires 379.2016.}

5-Benzylxoy-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.  

\[ ^1 \text{H NMR (270 MHz, CDCl}_3\]: } \delta = 2.31 \text{ (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 \text{ (3H, m), 7.24-7.43 ppm (6H, m). LC/MS (ES+): } m/z \text{ 363.3 (M}^+\text{+H). HRMS (ES): m/z found 363.2072; C}_{23}\text{H}_{27}\text{N}_2\text{O}_2^+ (M}^+\text{+H) requires 363.2067.}

5-Benzylxoy-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\(_3\)): \(\delta = 3.72 (2\text{H}, \text{s}), 3.79 (3\text{H}, \text{s}), 3.86 (2\text{H}, \text{s}), 4.34 (2\text{H}, \text{s, br}), 5.09 (2\text{H}, \text{s}), 6.28 (1\text{H}, \text{s}), 6.62 (1\text{H}, \text{s}), 7.17-7.43 \text{ ppm (9H, m)}. \) 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-Benzzyloxy-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\(_3\)): \(\delta = 3.72 (2\text{H}, \text{s}), 3.79 (3\text{H}, \text{s}), 3.82 (2\text{H}, \text{s}), 5.09 (2\text{H}, \text{s}), 6.27 (1\text{H}, \text{s}), 6.61 (1\text{H}, \text{s}), 6.98-7.12 (2\text{H}, \text{m}), 7.19-7.43 \text{ ppm (7H, m)}. \) 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-Benzzyloxy-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\(_3\)): \(\delta = 3.35 (1\text{H}, \text{s, br}), 3.72 (2\text{H}, \text{s}), 3.74 (2\text{H}, \text{s}), 3.79 (3\text{H}, \text{s}), 5.09 (2\text{H}, \text{s}), 6.28 (1\text{H}, \text{s}), 6.61 (1\text{H}, \text{s}), 7.15-7.43 \text{ ppm (9H, m)}. \) LC/MS (ES+): \(m/z\) 413.3 (M\(^+\)+H).

5-Benzzyloxy-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. ¹H NMR (270 MHz, CDCl₃): 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; C₂₄H₂₉N₂O₄⁺ (M⁺+H) requires 409.2122.

5-Benzyl-oxo-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. ¹H NMR (270 MHz, CDCl₃): 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; C₂₄H₂₉N₂O₄⁺ (M⁺+H) requires 409.2122.

5-Benzyl-oxo-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%). ¹H NMR (270 MHz, CDCl₃): 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; C₂₅H₃₁N₂O₅⁺ (M⁺+H) requires 439.2227.
3-Benzyl-7-benzyloxy-6-methoxy-3,4-dihydroquinazolin-2(1H)-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 CHCl₃ (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 (CH₂Cl₂→CH₂Cl₂/MeOH 10:1) afforded compound 5a as a yellow powder (220 mg, 49%); mp 186-188 °C. ¹H NMR (270 MHz, CDCl₃): 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. (C₂₃H₂₂N₂O₃) C, H, N.

7-Benzyloxy-6-methoxy-3-(2-methoxybenzyl)-3,4-dihydroquinazolin-2(1H)-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 Et₂O, filtered and dried to afford compound 5b as a white powder (250 mg, 33%); mp 177-178 °C. ¹H NMR (270 MHz, CDCl₃): 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; C₂₄H₂₄N₂O₄⁺ (M⁺+H) requires 405.1809. Anal. (C₂₄H₂₄N₂O₄·0.5H₂O) C, H, N.

7-Benzyloxy-6-methoxy-3-(3-methoxybenzyl)-3,4-dihydroquinazolin-2(1H)-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 Et₂O, filtered and dried to afford compound 5c as a yellow powder (340 mg, 43%); mp 136-138 °C. ¹H 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$_2$O$_4$+ (M$^+$+H) requires 405.1809. Anal. (C$_{24}$H$_{24}$N$_2$O$_4$ 0.5H$_2$O) C, H, N.

7-Benzylxoy-6-methoxy-3-(4-methoxybenzyl)-3,4-dihydroquinazolin-2(1H)-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$_2$O, 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$_2$O$_4$+ (M$^+$+H) requires 405.1809. Anal. (C$_{24}$H$_{24}$N$_2$O$_4$ 0.5H$_2$O) C, H, N.

7-Benzylxoy-6-methoxy-3-(2-methylbenzyl)-3,4-dihydroquinazolin-2(1H)-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$_2$Cl$_2$→CH$_2$Cl$_2$/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$_{24}$N$_2$O$_3$+ (M$^+$+H) requires 389.1860. Anal. (C$_{24}$H$_{24}$N$_2$O$_3$) C, H, N.

7-Benzylxoy-3-(2-chlorobenzyl)-6-methoxy-3,4-dihydroquinazolin-2(1H)-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 CHCl₃ (100 mL). The organic layer was filtered and concentrated in vacuo. Flash column chromatography (CH₂Cl₂→CH₂Cl₂/EtOAc 8:1) afforded compound 5f as an off-white powder (850 mg, 21%); mp 190-192 °C. ¹H NMR (270 MHz, CDCl₃): δ = 3.76 (3H, s), 4.27 (2H, s), 4.71 (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; C₂₃H₂₂ClN₂O₃⁺ (M⁺+H) requires 409.1313. Anal. (C₂₃H₂₁ClN₂O₃) C, H, N.

7-Benzylxoy-3-(2-fluorobenzyl)-6-methoxy-3,4-dihydroquinazolin-2(1H)-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 CHCl₃ (100 mL). The organic layer was filtered and concentrated in vacuo. Flash column chromatography (CH₂Cl₂→CH₂Cl₂/EtOAc 5:1) afforded compound 5g as an off-white powder (1.1 g, 29%); mp 209-211 °C. ¹H NMR (270 MHz, DMSO-d₆): 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; C₂₂H₂₂FN₂O₃⁺ (M⁺+H) requires 393.1609. Anal. (C₂₃H₂₁FN₂O₃) C, H, N.

7-Benzylxoy-3-(3-chlorobenzyl)-6-methoxy-3,4-dihydroquinazolin-2(1H)-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 CHCl₃ (100 mL). The organic layer was filtered and concentrated in vacuo. Flash column chromatography (CH₂Cl₂→CH₂Cl₂/EtOAc 4:1) afforded compound 5h as an off-white powder (1.35 g, 37%); mp 158-159 °C. ¹H NMR (270 MHz, CDCl₃): 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; C₂₃H₂₂ClN₂O₃⁺ (M⁺+H) requires 409.1313. Anal. (C₂₃H₂₁ClN₂O₃) C, H, N.
7-Benzzyloxy-6-methoxy-3-(2,3-dimethoxybenzyl)-3,4-dihydroquinazolin-2(1H)-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 CHCl₃ (100 mL). The organic layer was filtered and concentrated in vacuo. Flash column chromatography (CH₂Cl₂→CH₂Cl₂/EtOAc 1:1) afforded compound 5i as a white powder (780 mg, 25%); mp 166-168 °C. ¹H NMR (270 MHz, CDCl₃): 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; C₂₅H₂₇N₂O₅⁺ (M⁺+H) requires 435.1914. Anal. (C₂₅H₂₆N₂O₅) C, H, N.

7-Benzzyloxy-3-(2,5-dimethoxybenzyl)-6-methoxy-3,4-dihydroquinazolin-2(1H)-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 CHCl₃ (100 mL). The organic layer was filtered and concentrated in vacuo. Flash column chromatography (CH₂Cl₂→CH₂Cl₂/EtOAc 1:2) afforded compound 5j as an off-white powder (630 mg, 26%); mp 160-162 °C. ¹H NMR (270 MHz, CDCl₃): 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; C₂₅H₂₇N₂O₅⁺ (M⁺+H) requires 435.1914. Anal. (C₂₅H₂₆N₂O₅) C, H, N.

7-Benzzyloxy-6-methoxy-3-(3,4,5-trimethoxybenzyl)-3,4-dihydroquinazolin-2(1H)-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,
3-Benzyl-7-hydroxy-6-methoxy-3,4-dihydroquinazolin-2(1H)-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\(_3\)): \(\delta = 3.56 (2H, \text{s, br}), 3.81 (3H, \text{s}), 4.30 (2H, \text{s}), 4.65 (2H, \text{s}), 6.35 (1H, \text{s}), 6.47 (1H, \text{s}), 7.30-7.42 \text{ ppm (5H, m)}\). LC/MS (ES+): \(m/z\) 285.0 (M\(^+\)+H). HRMS (ES): \(m/z\) found 285.1232; \(\text{C}_{16}\text{H}_{17}\text{N}_{2}\text{O}_{3}\) (M\(^+\)+H) requires 285.1234. Anal. (C\(_{16}\)H\(_{16}\)N\(_2\)O\(_3\) 0.25H\(_2\)O) C, H, N.

7-Hydroxy-6-methoxy-3-(2-methoxybenzyl)-3,4-dihydroquinazolin-2(1H)-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\(_2\)O/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\(_3\)OD 5:1): \(\delta = 3.26 (2H, \text{s, br}), 3.70 (3H, \text{s}), 3.77 (3H, \text{s}), 4.24 (2H, \text{s}), 4.57 (2H, \text{s}), 6.24 (1H, \text{s}), 6.37 (1H, \text{s}), 6.78-6.89 (2H, \text{m}), 7.13-7.22 \text{ ppm (2H, m)}\). LC/MS (ES+): \(m/z\) 315.1 (M\(^+\)+H). HRMS (ES): \(m/z\) found 315.1340; \(\text{C}_{17}\text{H}_{19}\text{N}_{2}\text{O}_{4}\) (M\(^+\)+H) requires 315.1339. Anal. (C\(_{17}\)H\(_{18}\)N\(_2\)O\(_4\) 0.25H\(_2\)O) C, H, N.

7-Hydroxy-6-methoxy-3-(3-methoxybenzyl)-3,4-dihydroquinazolin-2(1H)-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 Et₂O/EtOAc (1:1), filtered and dried to afford compound 6c as an off-white powder (155 mg, 87%); mp 197-199 °C. ¹H NMR (270 MHz, CDCl₃/CD₃OD 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; C₁₇H₁₉N₂O₄⁺ (M⁺+H) requires 315.1339. Anal. (C₁₇H₁₈N₂O₄ 0.25H₂O) C, H, N.

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

Method as for 6a using compound 5d (180 mg, 0.44 mmol) and Pd/C (10%, 30 mg) in THF (10 mL) and MeOH (20 mL) under hydrogen at rt for 18 h. The residue was stirred in Et₂O/EtOAc (10:1), filtered and dried to afford compound 6d as a white powder (110 mg, 79%); mp 175-177 °C. ¹H NMR (270 MHz, CDCl₃/CD₃OD 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; C₁₇H₁₉N₂O₄⁺ (M⁺+H) requires 315.1339. Anal. (C₁₇H₁₈N₂O₄ 0.25H₂O) C, H, N.

7-Hydroxy-6-methoxy-3-(2-methylbenzyl)-3,4-dihydroquinazolin-2(1H)-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. ¹H NMR (270 MHz, DMSO-d₆): 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; C₁₇H₁₉N₂O₃⁺ (M⁺+H) requires 299.1390. Anal. (C₁₇H₁₈N₂O₃ 1.5H₂O) C, H, N.
3-(2-Chlorobenzyl)-7-hydroxy-6-methoxy-3,4-dihydroquinazolin-2(1H)-one 6f

Compound 5f (350 mg, 0.86 mmol) and methanesulfonic acid (2.5 mL) were stirred in CH₂Cl₂ (2.5 mL) at rt for 24 h. NaHCO₃ (sat.) was added, and the mixture was extracted with EtOAc. The organic layer was washed with water, brine and dried (MgSO₄), filtered and concentrated in vacuo. Flash column chromatography (PE→EtOAc) afforded compound 6f as a white powder (100 mg, 37%); mp 208-210 °C. ¹H NMR (270 MHz, DMSO-d₆): 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; C₁₆H₁₅ClN₂O₃⁺ (M⁺+H) requires 319.0844. Anal. (C₁₆H₁₅ClN₂O₃) C, H, N.

3-(2-Fluorobenzyl)-7-hydroxy-6-methoxy-3,4-dihydroquinazolin-2(1H)-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. ¹H NMR (270 MHz, DMSO-d₆): 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; C₁₆H₁₅FN₂O₃⁺ (M⁺+H) requires 303.1139. Anal. (C₁₆H₁₅FN₂O₃) C, H, N.

3-(3-Chlorobenzyl)-7-hydroxy-6-methoxy-3,4-dihydroquinazolin-2(1H)-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. ¹H NMR (270 MHz, DMSO-d₆): 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(1H)-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. 1H 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(1H)-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. 1H 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(1H)-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\textsubscript{3}): \(\delta = 3.78\) (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\textsubscript{19}H\textsubscript{23}N\textsubscript{2}O\textsubscript{6}\textsuperscript{+} (M\(^+\)+H) requires 375.1551. Anal. (C\textsubscript{19}H\textsubscript{22}N\textsubscript{2}O\textsubscript{6} 0.5H\textsubscript{2}O) 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\textsubscript{4}), filtered and concentrated in vacuo. The residue was stirred in Et\textsubscript{2}O, 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\textsubscript{3}/CD\textsubscript{3}OD 1:1): \(\delta = 3.73\) (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\textsubscript{16}H\textsubscript{18}N\textsubscript{3}O\textsubscript{5}S\textsuperscript{+} (M\(^+\)+H) requires 364.0962. Anal. (C\textsubscript{16}H\textsubscript{17}N\textsubscript{3}O\textsubscript{5}S 0.5H\textsubscript{2}O) 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\textsubscript{2}O, filtered and dried to afford compound 7b as a white powder (70 mg, 71%); mp 160-161 °C. \(^1\)H NMR (270 MHz, DMSO-\textsubscript{d}6): \(\delta = 3.71\) (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\textsubscript{17}H\textsubscript{20}N\textsubscript{3}O\textsubscript{6}S\textsuperscript{+} (M\(^+\)+H) requires 394.1067. Anal. (C\textsubscript{17}H\textsubscript{19}N\textsubscript{3}O\textsubscript{6}S 0.5H\textsubscript{2}O) C, H, N.
6-Methoxy-3-(3-methoxybenzyl)-7-sulfamoyloxy-3,4-dihydroquinazolin-2(1H)-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 Et₂O, filtered and dried to afford compound 7c as a white powder (95 mg, 76%); mp 185-187 °C. ¹H NMR (270 MHz, DMSO-δ6): 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; C₁₇H₂₀N₃O₆S⁺ (M⁺+H) requires 394.1067. Anal. (C₁₇H₁₉N₃O₆S 0.5H₂O) C, H, N.

6-Methoxy-3-(4-methoxybenzyl)-7-sulfamoyloxy-3,4-dihydroquinazolin-2(1H)-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 Et₂O, filtered and dried to afford compound 7d as a white powder (70 mg, 71%); mp 123-125 °C. ¹H NMR (270 MHz, DMSO-δ6): 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; C₁₇H₂₀N₃O₆S⁺ (M⁺+H) requires 394.1067. Anal. (C₁₇H₁₉N₃O₆S 0.5H₂O) C, H, N.

6-Methoxy-3-(2-methylbenzyl)-7-sulfamoyloxy-3,4-dihydroquinazolin-2(1H)-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 Et₂O/EtOAc (1:1), filtered and dried to afford compound 7e as a white powder (110 mg, 73%); mp 181-183 °C. ¹H NMR (270 MHz, DMSO-δ6): 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; C₁₇H₂₀N₃O₅S⁺ (M⁺+H) requires 378.1118. Anal. (C₁₇H₁₉N₃O₅S) C, H, N.
3-(2-Chlorobenzyl)-6-methoxy-7-sulfamoyloxy-3,4-dihydroquinazolin-2(1H)-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-$d_6$): $\delta = 3.69$ (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$_3$O$_5$S$^+$ (M$^+$+H) requires 399.0572. Anal. (C$_{16}$H$_{16}$ClN$_3$O$_5$S) C, H, N.

3-(2-Fluorobenzyl)-6-methoxy-7-sulfamoyloxy-3,4-dihydroquinazolin-2(1H)-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$_2$O/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-$d_6$): $\delta = 3.69$ (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$_3$O$_5$S$^+$ (M$^+$+H) requires 382.0867. Anal. (C$_{16}$H$_{16}$FN$_3$O$_5$S) C, H, N.

3-(3-Chlorobenzyl)-6-methoxy-7-sulfamoyloxy-3,4-dihydroquinazolin-2(1H)-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$_2$O, filtered and dried to afford compound **7h** as a white powder (95 mg, 77%); mp 143-145 °C. $^1$H NMR (270 MHz, DMSO-$d_6$): $\delta = 3.69$ (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$_3$O$_5$S$^+$ (M$^+$+H) requires 399.0572. Anal. (C$_{16}$H$_{16}$ClN$_3$O$_5$S) C, H, N.
3-(2,3-Dimethoxybenzyl)-7-sulfamoyloxy-6-methoxy-3,4-dihydroquinazolin-2(1H)-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 Et₂O/EtOAc (1:1), filtered and dried to afford compound 7i as a white powder (95 mg, 64%); mp 164-166 °C. ¹H NMR (270 MHz, DMSO-d₆): 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; C₁₈H₂₂N₃O₇S⁺ (M⁺+H) requires 424.1173. Anal. (C₁₈H₂₁N₃O₇S) C, H, N.

3-(2,5-Dimethoxybenzyl)-7-sulfamoyloxy-6-methoxy-3,4-dihydroquinazolin-2(1H)-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 Et₂O/EtOAc (1:1), filtered and dried to afford compound 7j as a white powder (95 mg, 77%); mp 176-178 °C. ¹H NMR (270 MHz, DMSO-d₆): 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; C₁₈H₂₂N₃O₇S⁺ (M⁺+H) requires 424.1173. Anal. (C₁₈H₂₁N₃O₇S) C, H, N.

6-Methoxy-7-sulfamoyloxy-3-(3,4,5-trimethoxybenzyl)-3,4-dihydroquinazolin-2(1H)-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 Et₂O, filtered and dried to afford compound 7k as a cream colored solid (95 mg, 78%); mp 112-114 °C. ¹H NMR (270 MHz, DMSO-d₆): 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; C₁₀H₂₄N₂O₈S⁺ (M⁺+H) requires 454.1279. Anal. (C₁₀H₂₃N₃O₈S) 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](http://pubs.ac.org). ¹³C 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 T₂R-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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**Abbreviations Used:** DHQ, dihydroquinazolinone; THIQ, tetrahydroisoquinoline; STS, steroid sulfatase; FGly, formylglycine; CAIX, carbonic anhydrase IX.

**References**


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