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1-Alkyl-4-phenyl-6-alkoxy-1*H*-quinazolin-2-ones: A Novel Series of Potent Calcium-Sensing Receptor Antagonists

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Parathyroid hormone (PTH) is an effective bone anabolic agent. However, only when administered by daily sc injections exposure of short duration is achieved, a prerequisite for an anabolic response. Instead of applying *exogenous* PTH, mobilization of *endogenous* stores of the hormone can be envisaged. The secretion of PTH stored in the parathyroid glands is mediated by a calcium sensing receptor (CaSR) a GPCR localized at the cell surface. Antagonists of CaSR (calcilytics) mimic a state of hypocalcaemia and stimulate PTH release to the bloodstream. Screening of the internal compound collection for inhibition of CaSR signaling function afforded **2a**. In vitro potency could be improved >1000 fold by optimization of its chemical structure. The binding mode of our compounds was predicted based on molecular modeling and confirmed by testing with mutated receptors. While the compounds readily induced PTH release after iv application a special formulation was needed for oral activity. The required profile was achieved by using microemulsions. Excellent PK/PD correlation was found in rats and dogs. High levels of PTH were reached in plasma within minutes which reverted to baseline in about 1−2 h in both species.

Introduction

Osteoporosis is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue that leads to bone fragility and increased risk of fractures. The disease is associated with high cost for the health care system and substantial morbidity and mortality especially after hip fractures. In osteoporotic individuals the delicate equilibrium between bone formation by osteoblasts and resorption by osteoclasts is out of balance leading to progressive bone loss. Osteoporosis could largely be prevented by treatment with one of the commercially available resorption inhibitors (e.g., a bisphosphonate). However, many patients are only diagnosed at a time when they have already lost a considerable amount of bone mass. There is consequently a clear need for bone anabolic agents which can rebuild lost bone. The only bone anabolics currently on the market are based on the natural hormone parathyroid hormone (PTH[a]), a polypeptide consisting of 84 amino acids. The N-terminal fragment PTH(1−34) (teriparatide) is approved in the U.S. as well as in Europe while the full length peptide PTH(1−84) is only available in Europe. Administration of these peptides is so far only possible by daily subcutaneous (sc) injections which is not ideal for a chronic therapy.[1] Stimulation of the release of *endogenous* PTH from parathyroid glands therefore would provide an attractive alternative.

PTH secretion is controlled by a calcium-sensing receptor (CaSR), a member of the GPCR family 3, expressed on the surface of parathyroid cells.[2] Extracellular Ca^{2+} concentrations below the normal physiological level stimulate PTH release, while at high [Ca^{2+}] little or no PTH is secreted. With a CaSR modulator PTH levels can be controlled independently of extracellular [Ca^{2+}]. Calcimimetic compounds (agonists of CaSR) activate the receptor and inhibit PTH secretion, while calcilytics (antagonists) mimic a state of hypocalcaemia and thus stimulate the release of endogenous PTH.[3] However, it is well documented that elevated levels of PTH only result in higher bone mass if they are transient, i.e., they do not persist for more than about 2−4 h.[4] Elevated PTH levels sustained for several hours activate not only osteoblasts but also osteoclasts leading to an increase in bone turnover instead of higher bone mass.

Cinacalcet, a calcimimetic, was approved several years ago for the treatment of secondary hyperparathyroidism.[5] The first calcilytic studied in some detail was **1a** (NPS 2143)[6] with a β-amino-alcohol partial structure (Figure 1). Daily oral administration of **1a** to ovariectomized rats resulted in elevated PTH levels sustained for several hours (>4 h). Higher bone turnover was consequently observed rather than an anabolic net increase in bone mass. Only by blocking the increased bone resorption with the antiresorptive agent 17β-estradiol a

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[a] Abbreviations: 7TM, seven-transmembrane; BMD, bone mineral density; CaSR, calcium sensing receptor; DMEU, 1,3-dimethyl-2-imidazolidinone; EDTA, ethylenediaminetetraacetic acid; FLIPR, fluorimetric calcium assay; GPCR, G-protein-coupled receptor; iv, intravenous; MEPC, microemulsion preconcentrate; PAMPA, parallel artificial membrane permeability; PD, pharmacodynamic; PK, pharmacokinetic; po, oral; PTH, parathyroid hormone; SAR, structure−activity-relationship; sc, subcutaneous; TFA, trifluoroacetic acid; h, hour; rt, room temperature; mp, melting point; HV, high vacuum; equiv, equivalent, quint, 5-line system, hex, 6-line system, hept, 7-line system.

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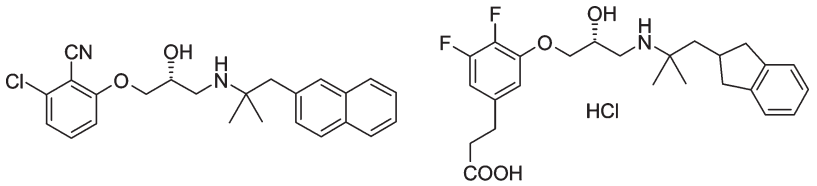
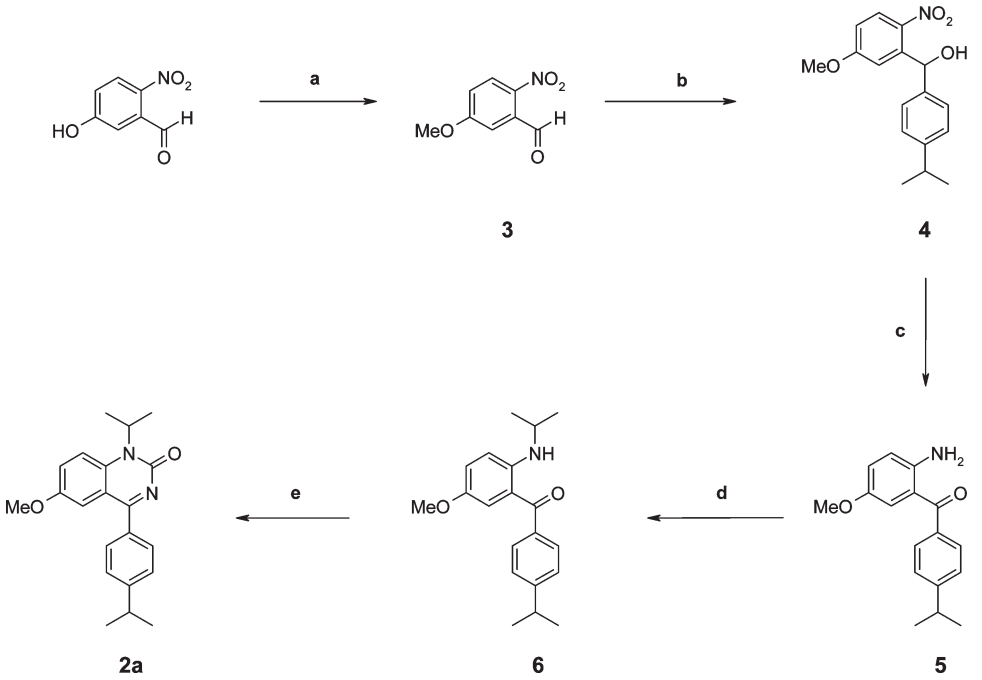


1a 1b

Figure 1. Structures of calcilytics **1a** and **1b**.

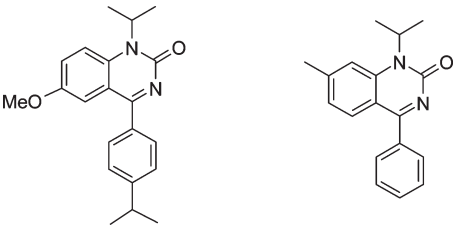
Scheme 1. Synthesis of 1-Isopropyl-4-(4-isopropyl-phenyl)-6-methoxy-1*H*-quinazolin-2-one[a]



[a] Reagents and conditions: (a) methyl iodide, K_2CO_3, DMF, rt, 12 h; (b) 4-isopropyl-phenyl-magnesium bromide, THF; −78 °C, 2 h; (c) (i) Jones-reagent; acetone, 0 °C, 1 h, (ii) H_2, RaNi, rt, atmospheric pressure, MeOH/THF 1:1; (d) isopropyl iodide, K_2CO_3, 110 °C, 48 h; (e) sodium cyanate, AcOH, rt, overnight.

gain in bone mineral density (BMD) could be achieved. The follow-up compound **1b** (Ronacaleret; SB-751689)[7] with a similar structure but a more appropriate PK profile was clinically tested (Figure 1). Proof of concept was achieved by showing significant increases in bone formation markers in postmenopausal women, while resorption markers remained at baseline levels.[8] More recently, other structural series of calcilytics have also been reported.[9−12]

The starting point for our calcilytics project was compound **2a** found by screening the internal Novartis compound collection for inhibition of CaSR signaling function using a fluorimetric assay for intracellular calcium (FLIPR). The structure is related to Proquazone[12,13] developed at Sandoz in the early 1980s as an analgesic (Figure 2). The compound had an IC_{50} of 1.45 μM and was an interesting starting point for lead optimization being a close analogue of a marketed compound.



2a Proquazone

Figure 2. Structures of lead compound **2a** and Proquazone.

Chemistry

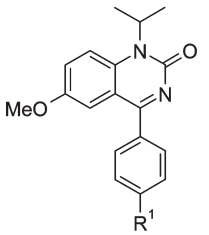
Scheme 1 outlines the synthesis of the lead compound **2a**. The commercially available 5-hydroxy-2-nitrobenzaldehyde was *O*-methylated and then treated with 4-isopropyl-phenyl-magnesium bromide at low temperature to yield the secondary alcohol **4**. Jones oxidation followed by catalytic reduction afforded the amino-ketone **5**. The synthesis was completed by monoalkylation of the aniline nitrogen followed by cyclization to the target compound **2a** by treatment with sodium cyanate in glacial acetic acid.

Derivatives with various 4-substituents at the phenyl group (cf. Table 1) were easily accessible using the same synthetic sequence employing the appropriate Grignard reagents.

The methylether of **2a** could be cleaved with boron tribromide to the give **7a** with a free phenol group. Alkylation with a range of bromides in DMEU (1,3-dimethyl-2-imidazolidinone) in the presence of cesium carbonate gave easy access to a range of analogues with different ether functionalities (cf. Scheme 2 and Table 2).

1-Benzyl-4-(4-isopropyl-phenyl)-6-propargyloxy-(1*H*)-quinazolin-2-ones (**10**, cf. Scheme 3 and Table 3) were prepared similarly to the reaction sequence depicted in Scheme 1 starting

Table 1. SAR of the *p*-Substituent of the 4-Phenyl Group

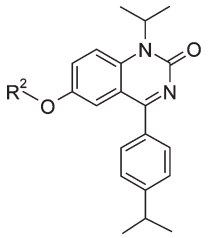


2

compound	R^1	CaSR FLIPR assay IC_{50} [μM][a]
2a	^{i}Pr	1.4
2b	H	> 30
2c	Me	> 30
2d	Et	2.1
2e	cyclo-Pr	3.3
2f	^{t}Bu	2.1
2g	Ph	5.5
2h	O^{i}Pr	> 30
2i	COOMe	> 30
2j	COOH	> 30

[a] Average of two or more runs. Standard deviation of assay is generally less than ±40%.

Table 2. SAR of the 6-Alkoxy Substituent

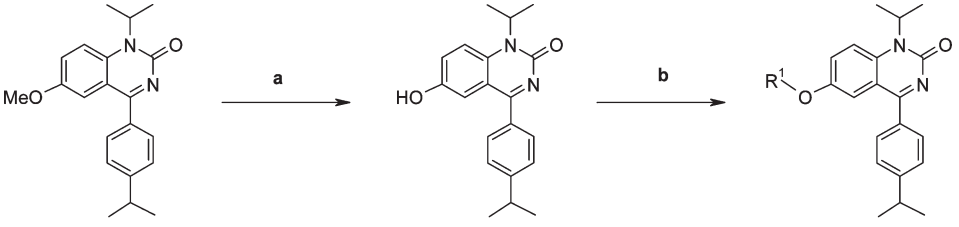


7

compound	R^2	CaSR FLIPR-assay IC_{50} [μM][a]
2a	Me	1.4
7a	H	15.9
7b	Et	2.2
7c	Pr	1.1
7d	^{i}Pr	2.7
7e	Bu	3.7
7f	allyl	0.18
7g	methallyl	6.6
7h	propargyl	0.009
7i	2-butynyl	0.17

[a] Average of two or more runs. Standard deviation of assay is generally less than ±40%.

Scheme 2. Modification of the Ether Functionality[a]



2a **7a** **7b-i**

[a] Reagents and conditions: (a) BBr_3, CH_2Cl_2, rt, 1 h; (b) R^1-Br, Cs_2CO_3, DMEU, rt, 18 h.

from 2-nitro-5-hydroxy-benzaldehyde (cf. Experimental Procedures). For the reduction of the nitro group iron powder in acetic acid was used instead of catalytic hydrogenation to preserve the alkyne moiety. The N-(1) substituent was introduced either by alkylation of **8** with an appropriately substituted benzyl bromide or by reductive amination with the corresponding benzaldehyde (Scheme 3). Cyclization to the quinazolinone heterocycles **10** was accomplished by reaction with sodium cyanate. Thio analogues **11** were prepared by ring closure with benzoyl isothiocyanate.

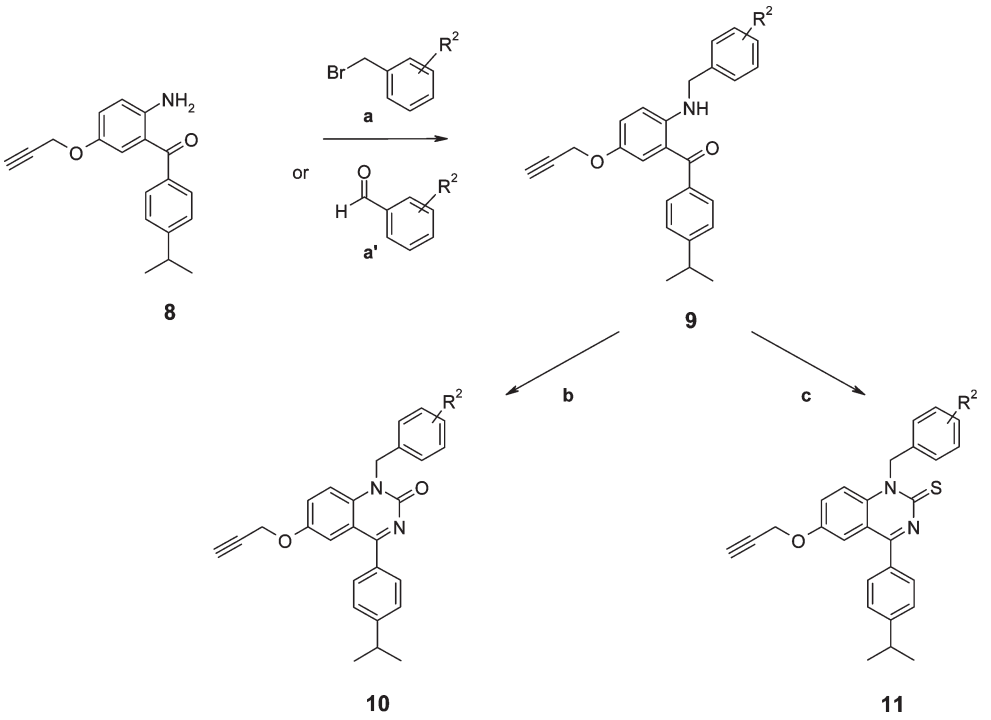
The synthesis of quinazolinones with 6-amino groups started with 2-chloro-5-nitro-benzaldehyde (Scheme 4). Standard treatment with 4-isopropyl-phenyl magnesium bromide and Jones oxidation led to the ketone **12**. The chlorine was then replaced by isopropyl amine to afford **13**, which was cyclized with chlorosulfonyl isocyanate to **14**. Catalytic reduction with Raney nickel to aniline **15** and mono- or dialkylation completed the synthesis of derivatives **16** (Table 4).

When the aniline **17** was treated at 180 °C in a water/DMEU mixture under microwave conditions, sigmatropic rearrangement took place giving rise to phenol **18a** with a 2-allyl group (Scheme 5). The regioselectivity observed can be rationalized by the push pull system caused through the electron donating capacity of the amino group and the electron withdrawing character of the carbonyl moiety. Compound **18a** was benzylated (**19a**) and cyclized to the quinazolinone **20a**. Alternatively, the allyl group was derivatized, e.g., by hydrogenation (**19b**) prior to cyclization to **20b**. Pd catalyzed cyclopropanation of **18a** furnished **18c**, which was analogously transformed to **20c**.

Results and Discussion

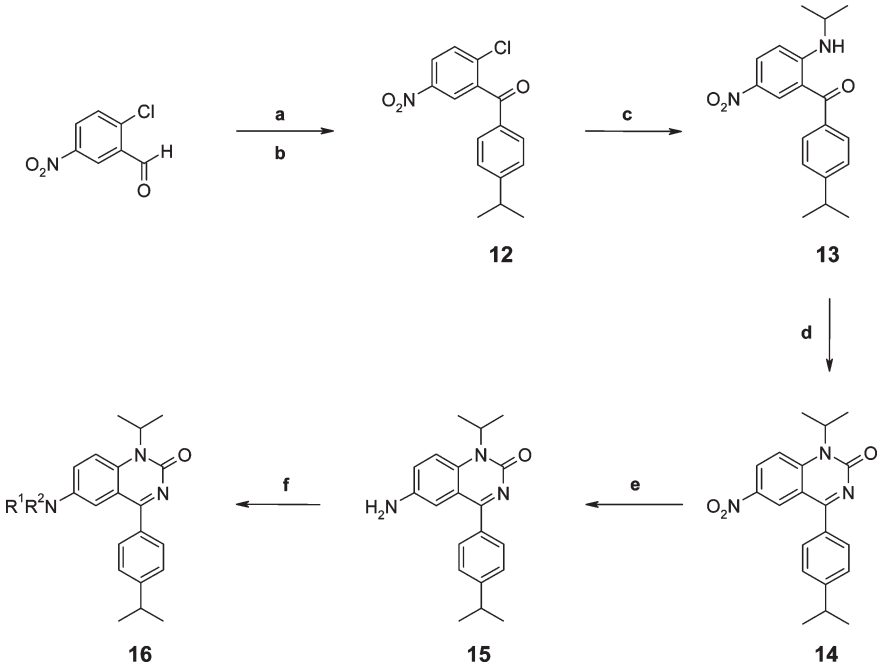
From Hit to Potent Calcilytics (SAR). A fluorimetric assay was used as a primary screen to determine inhibitory potency of the compounds against the CaSR. The assay measures

Scheme 3. Synthesis of 1-Benzyl-4-(4-isopropyl-phenyl)-6-propargyloxy-1*H*-quinazolin-2-ones[a]



[a] Reagents and conditions: (a) Huenig's base, 1.4-dioxane, 100 °C; (a′) $NaB(OAc)_3H/CH_2Cl_2$ or $NaCNBH_3$/MeOH, rt; (b) sodium cyanate, AcOH, rt; (c) benzoyl isothiocyanate, THF, rt.

Scheme 4. Synthesis of 6-Amino-4-(4-isopropyl-phenyl)-1-isopropyl-1*H*-quinazolin-2-one[a]

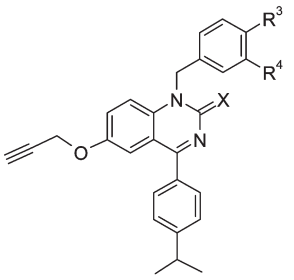


[a] Reagents and conditions: (a) 4-isopropyl-phenyl-magnesium bromide, THF; −75 °C → rt, 3 h; (b) Jones reagent; acetone, 0 °C; (c) isopropyl amine, 65 °C, 10 h; (d) chlorosulfonyl isocyanate, 0 °C; (e) H_2, Ra−Ni, EtOH/THF; (f) cf. Experimental Procedures below.

calcium mobilization in hamster fibroblasts transfected with the human receptor.

Table 1 shows that the 4-isopropyl group at the phenyl residue is important for antagonistic activity. Both the hydrogen (**2b**) or the methyl (**2c**) analogues show only marginal activity. However, 4-isopropyl may be replaced by other alkyl substituents, such as an ethyl (**2d**), cyclopropyl (**2e**) or *t*-butyl (**2f**) group without affecting potency significantly. A phenyl group is somewhat tolerated (**2g**) but not residues containing functional groups such as an ester (**2i**) or an acid (**2j**). An isopropoxy substituent (**2h**) is similarly detrimental to potency. A short lipophilic substituent is thus preferred in this position. The *p*-isopropyl group was moved to the *m*- and *o*-positions, but this resulted in clearly less potent

Table 3. SAR of Substituted *N*(1)-Benzyl Derivatives

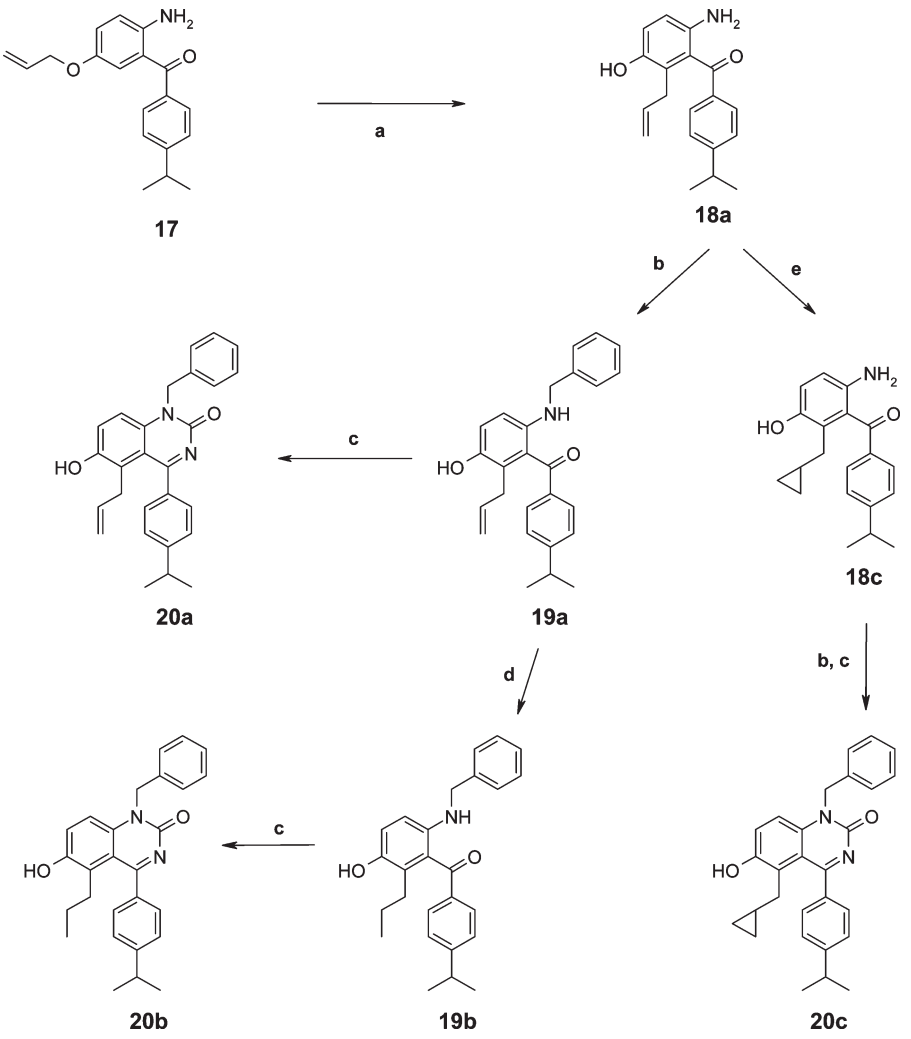


X = O **10**
S **11**

compound	R^3	R^4	CaSR FLIPR assay IC_{50} [nM][a]
10a	H	H	4.4
10b	Me	H	1.6
10c	Et	H	4.2
10d	^{i}Pr	H	27
10e	OMe	H	4.2
10f	OEt	H	19
10g	OCH_2COOH	H	12.6
10h	H	Me	1.8
10i	H	Et	13
10j	H	OMe	2.7
10k	OMe	OEt	1.4
10l	H	OCH_2COOH	3.4
10m	H	OCH_2CH_2OH	7.3
11m	H	OCH_2CH_2OH	0.4

[a] Average of two or more runs. Standard deviation of assay is generally less than ±40%.

Scheme 5. Synthesis of 6-Hydroxy-1*H*-quinazolin-2-ones[a]

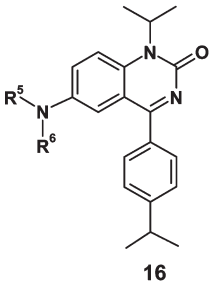


[a] Reagents and conditions: (a) autoclave, DMEU/water, 165 °C, 48 h or microwave, 180 °C (small scale); (b) benzaldehyde, sodium cyanoborohydride, AcOH/CH_2Cl_2, rt; (c) sodium cyanate, AcOH, rt, overnight; (d) H_2, Ra−Ni, rt, 2 h; (e) diazomethane, Pd(II)acetate, rt, 16 h.

compounds (data not shown). Addition of a second substituent on the phenyl ring had little or no effect (data not shown).

The nature of the alkoxy substituent in the 6-position was found to have a pronounced effect on potency. While the analogue with a free phenolic group (**7a**) was about 10 times

Table 4. SAR of 6-Amino-Substituted Quinazolinones



16

compound	R^5	R^6	CaSR FLIPR assay IC_{50} [μM][a]
16a	Et	H	0.46
16b	Et	Et	0.52
16c	Pr	H	1.1
16d	Pr	Pr	2.9
16e	allyl	H	0.07
16f	allyl	Me	0.23
16g	propargyl	H	0.03
16h	propargyl	propargyl	0.12
16i	benzyl	H	10

[a] Average of two or more runs. Standard deviation of assay is generally less than ±40%.

less potent than the lead **2a** short alkoxy groups with up to a C_4-alkyl chain all showed similar IC_{50} values (see compounds **7b**−**e**, Table 2). Improved potencies were obtained for the allyloxy and especially the propargyloxy derivative (see **7f** and **7h**, respectively). These groups seem to fit into a specific apolar binding pocket very tightly because already slightly larger groups such as methallyl (**7g**) or 2-butynyl (**7i**) caused some loss in activity. Small substituents (like H or Me) seem to lack the required attractive van der Waals forces while large and/or polar groups are not tolerated in this position. Replacement of the propargyl group by common C−C triple bond replacers was not successful (data not shown in the table). Such derivatives were either less potent (analogues with an *O*-benzyl moiety) or had unfavorable PK properties (replacement of the propargyl by an acetonitrile group).

The part of the molecule which was tolerant to a wide range of structural variations was the *N*(1)-substituent. Isopropyl could be replaced by a large variety of alkyl groups which may be substituted further by additional functionalities. The most potent compounds, however, were those with a *N*(1)-benzyl group. A representative selection is presented in Table 3. With respect to the unsubstituted benzyl derivative **10a**, additional substituents in the *m*- or *p*-position increased the potency by a factor of 2−5. Little difference was found between the *m*- and *p*-subseries as long as the substituents were small. However, with growing length of the residues *m*-substitution was increasingly preferred (data not shown). Ortho substituents, on the other hand, were detrimental for potency. The addition of polar groups resulted in a substantial reduction of potency. However, most of the in vitro activity was maintained by linking such residues via a spacer as in derivatives **10g** and **10l** (cf. also Results and Discussion below for structures **21**). Unfortunately, our expectations that the increased polarity of **10l** would be beneficial for in vivo potency was not met (data not shown).

Replacement of the carbonyl group of the quinazolinone core by a thiocarbonyl unit resulted in the most potent derivatives of the series. Compound **11m** showed an IC_{50} of 0.4 nM, an increase by a factor of nearly 20 compared to its oxygen analogue **10m**. Overall, we have succeeded to improve the micromolar IC_{50} of our initial hit roughly by a factor of 1000 to arrive at derivatives with nanomolar potencies. The glycol moiety in compounds **10m** and **11m** was introduced to increase aqueous solubility of the very lipophilic structure (cf. Results and Discussion below).

Structural modifications of the core were not very successful, e.g., replacement of one of the ring nitrogens by a carbon atom leading to quinolinones reduced potency substantially. The same was found for derivatives with an enlarged heterocycle (diazepinones) or a smaller one (indoles, benzimidazolones or indazoles).

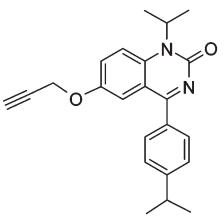
Improving Aqueous Solubility. As pointed out previously a calcilytic must not only be a potent antagonist of the CaSR but most importantly its PK profile must be appropriate in order to elicit a bone anabolic effect. The required parameters are high C_{max} at an early time point (T_{max}) and a short terminal half-life to ensure expeditious return of exposure and PTH levels to baseline.

All of the quinazolinone structures described so far are highly lipophilic (high clogP). It was therefore not surprising that only low exposure levels were observed after oral dosing with protracted return to baseline (cf. below). For an early C_{max} the gastrointestinal dissolution/absorption rate of the compounds had to be increased. The most straightforward method to achieve this is by way of improving their aqueous solubility. Unfortunately polar and/or solubilizing groups are not tolerated at most sites. This may be rationalized in terms of the assumed binding mode of our compounds. The local environment in the binding pocket is very lipophilic thus precluding the accommodation of polar residues without significant loss in antagonistic potency.

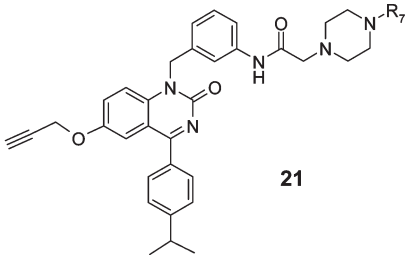
All efforts to introduce polar groups at the 4-phenyl substituent only led to derivatives with much decreased potency. In contrast, replacement of the 6-alkoxy substituent by mono- or dialkylamino groups (cf. Table 4) was tolerated. Similar SAR was found to the one established in the parent ether series (Table 2). *N*,*N*-Disubstituted derivatives are generally a little less potent compared to the monosubstituted ones, except for **16b** with two small ethyl groups. The best derivative was the one with a single *N*-propargyl substituent (**16g**; IC_{50} 30 nM) corroborating the SAR of the alkoxy series (cf. **7h** IC_{50} 9 nM). However, aqueous solubility of this subclass was still very limited because the anilinic nitrogen is only a very weak base. Derivative **16e** was tested in a PK experiment in rats. Only minimal exposure levels were found, mainly due to its high clearance.

Analogues with a free phenol group are accessible from the precursor **17** by thermal regioselective sigmatropic rearrangement of the 6-allyl group (cf. chemistry section). The 6-hydroxy-quinazolinones **20a**−**c** are very potent with IC_{50}

Table 5. Quinazolinones with Basic Benzyl Side Chains



7h



21

R^7		Me	$(CH_2)_2OMe$	$(CH_2)_3NMe_2$
compound	**7h**	**21a**	**21b**	**21c**
FLIPR assay IC_{50} [nM][a]	8.8	2.4	13	8.1
clogP	5.6	4.8	5.0	5.3
solubility [mg/mL] @ pH 6.8	0.02	0.02	0.02	0.1
solubility [mg/mL] @ pH 1.0	0.02	>0.2	>0.2	>0.2
PAMPA permeability[15]	high	medium	low	low

[a] Average of two or more runs. Standard deviation of assay is generally less than ±40%.

values of 3.9 (**20a**), 6.6 (**20b**), and 2.5 nM (**20c**). Interestingly propargylation of the phenolic OH-group of **20a** decreased potency by almost a factor of 100 in the fluorimetric calcium assay (FLIPR).

Unfortunately, polarity and hence aqueous solubility of these compounds were still not much improved. Not surprisingly, pharmacokinetic experiments of the most potent derivative **20c** again showed low exposure and low C_{max} values, believed to be mainly the consequence of high clearance caused by fast phase II metabolism of the free phenol group.

As already pointed out, the best site for modifications is the position at *N*(1). Substituents attached to the *m*- or *p*-position of the *N*(1)-benzyl group point toward the hydrophilic outside of the cell membrane according to the binding mode proposed in the modeling section. Thus it was envisaged that polar residues could be tolerated there without affecting calcilytic potency significantly. Out of a large series of derivatives containing basic amine groups attached to the benzyl moiety via different linkers and spacers of varying length, compounds of structure **21** were among the most promising ones (Table 5). Their clogP values, however, are not much different from that of **7h** (cf. Table 5). While **21a** and **21b** still only exhibited acceptable solubility at low pH derivative **21c** with an additional dimethyl-amino group did so even under neutral conditions. To our disappointment, however, the substantially improved aqueous solubility was not reflected in a better PK profile. On the contrary, pharmacokinetic measurements in the rat showed better oral bioavailability (ca. 20%) for **7h** than for all the piperazines **21** (only 1−2% for **21a** and **b** and about 8% for **21c**). C_{max} remained low for **21a** and **b**, but T_{max} was short and the return to baseline swift as desired for calcilytics. Peak levels for the third piperazine **21c** were higher, but elimination rather slow such that substantial exposure persisted throughout the measuring period of 6 h. The increased size of compounds **21** and the unequal distribution of polar surface area might offer an explanation for their poor oral bioavailability. Also, permeability was found to be reduced with respect to **7h** in an in vitro test system using artificial membranes[15] (PAMPA). These results made it rather obvious that simple addition of solubilizing polar groups may not be sufficient to make the current series of calcilytics orally active.

Docking of Compound 10a into a Homology Model of the Human CaSR. Various homology models for the calcium-sensing receptor (CaSR) have been proposed over the past few years.[16−20] While they all use the 7TM region of the crystal structure of bovine rhodopsin as a template, they differ in the primary sequence alignment for some TM helices. Despite the resulting differences in the detailed 3D structures, the models help to understand the consequences of mutations of residues considered relevant for ligand binding. Importantly, the combination of detailed models and point mutations clearly suggested that calcimimetics and calcilytics both bind in the 7TM domain and not in the extracellular part of the receptor.

Our initial homology model[17] had already suggested[20] that ligands with the quinazolinone core structure described in this work bind to a different region of the receptor than compounds like **1a**.

The binding mode shown in Figures 3 and 4 was obtained with our latest CaSR model,[19] docking compound **10a** manually into the receptor, after analyzing possible cavities large enough to accommodate this type of ligand. The complex was then adjusted by simple energy refinement (conjugate gradient minimization using the Amber force field with parameter sets *parm03*[22] for the protein and *gaf*[23] for the ligand were used).

The result is in agreement with mutation studies on the four residues shown in red or orange in Figure 3.

In our studies,[20] mutations of the red residues W818 (TM6) and Y825 (TM6) only affect the binding of quinazolinone-type ligands. Mutations of the yellow residues E837 (TM7), R680 (TM3), I841 (TM7), and F668 (TM2) have no effect on this type of ligand but have a strong negative effect for compounds like **1a**.[16−20] E837 in particular seems to be the major anchor point for positively charged ligands of that type. The orange F684 (TM3) and F688 (TM3) have both been found to affect the binding of the quinazolinone derivatives reported here as well as the amino-alcohol structures of the **1a** type compounds, i.e., they are at the boundary (or intersection) of distinct binding sites for these two different types of compounds.

In conclusion, the binding site for the compound class presented here is clearly distinct from the one suggested previously for **1a** and analogues. Figure 4 shows the clear separation of the residues interacting with **1a** (shown in yellow) from those that do with ligand **10a** (green).

In Vivo PTH Release. For a first in vivo proof of concept study, rats were dosed intravenously to avoid potential problems associated to slow and/or poor gastrointestinal absorption which could drastically reduce C_{max} and preclude significant PTH release. As anticipated all compounds tested triggered short and sharp PTH peaks and good in vitro/in vivo correlation was observed (Figure 5).

The first oral tests were made by administering the compounds in a mixture of ethanol/corn oil to dissolve the highly lipophilic compounds. When 3 mg/kg of **7h** was given to rats, the compound was quickly detected in the blood but only at low levels which persisted for more than 6h (Figure 6). Absorption is protracted under these conditions and the resulting PK profile not suitable to achieve a bone anabolic effect.

Major factors influencing C_{max} and peak width in the time−concentration diagram are dissolution/absorption rates and tissue distribution/elimination. The absorption profile and especially the maximal blood/plasma concentration (C_{max}) of compounds which can easily penetrate GI

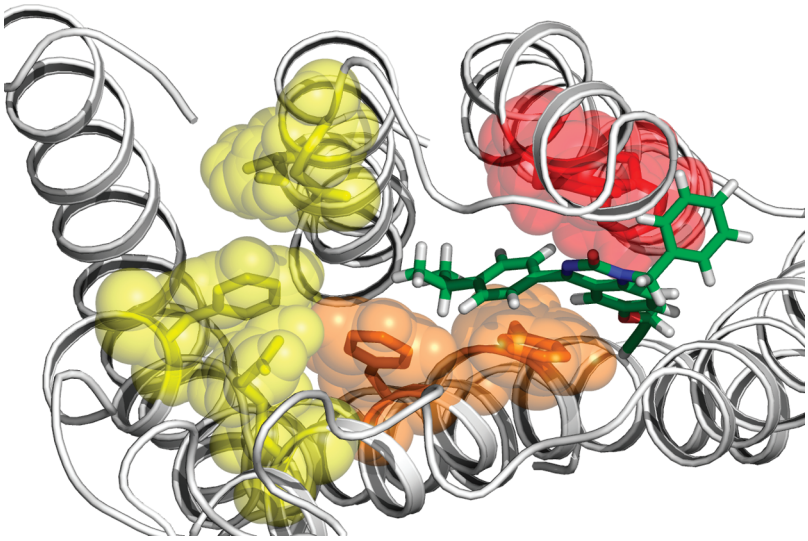


Figure 3. Proposed binding mode for **10a** (top view, from the extracellular area into the receptor). In red W818 (TM6) and Y825 (TM6) mutations, both of which affect the interactions with quinazolinone-type ligands; in yellow E837 (TM7), R680 (TM3), I841 (TM7), and F668 (TM2), which do not seem to be involved in binding of this type of ligands but clearly interfere with compounds like **1a**; in orange F684 (TM3) and F688 (TM3), which are supposed to interact with both types of ligands. (Figure made with PyMOL[21]).

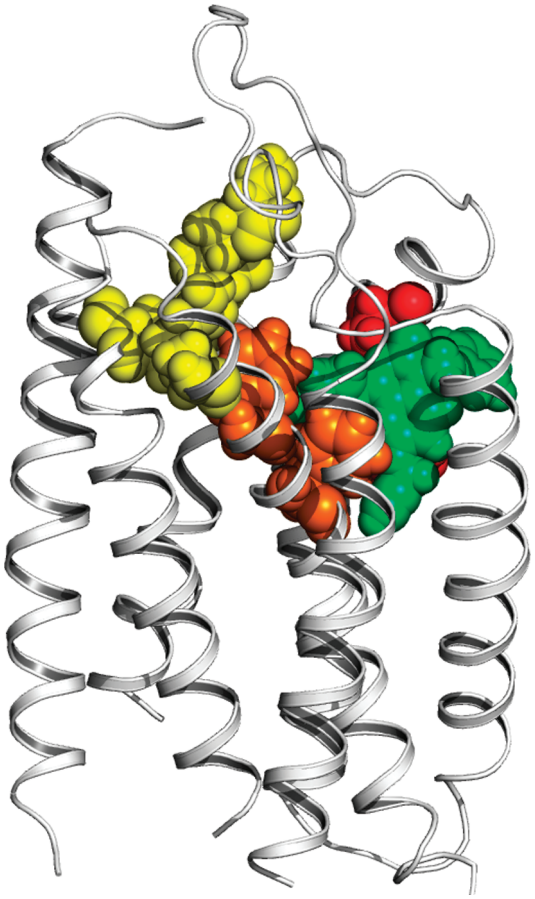


Figure 4. Side view of binding site (extracellular region on top). The ligand **10a** is represented in green, see Figure 3 and text for the further color coding. (Figure made with PyMOL[21]).

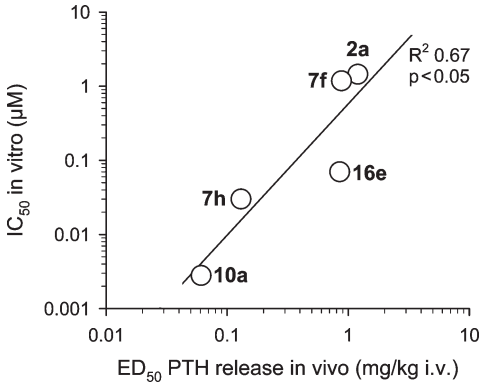


Figure 5. Correlation between in vitro and in vivo iv potency.

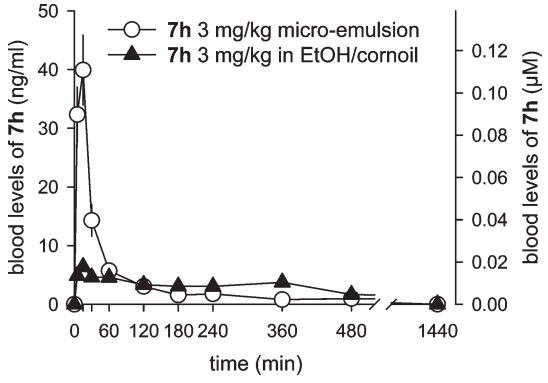


Figure 6. Comparison of blood levels of **7h** in rats after 3 mg/kg po administration using EtOH/corn oil or a microemulsion formulation.

epithelia but which show limited solubility/slow dissolution rate can often be improved significantly by an optimized pharmaceutical formulation. Molecules with such physicochemical properties are classified as class II compounds (according to the Biopharmaceutics Classification System of the FDA)[24] and the calcilytics of our lead series are typical examples.

Derivative **7h** was selected to be tested in rats using a range of different formulations known to improve PK behavior of class II compounds. Microemulsions were found to result in the best PK profiles, and this formulation principle was subsequently used for all further oral experiments. In strik-

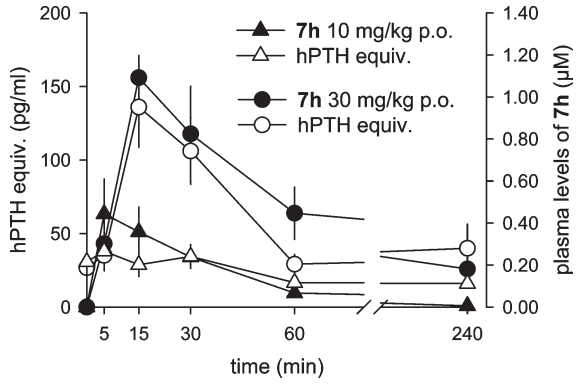


Figure 7. Plasma and bioactive parathyroid hormone (PTH, expressed as human PTH equivalents) levels after oral administration of a microemulsion formulation of **7h** at a dose of 10 and 30 mg/kg to rats.

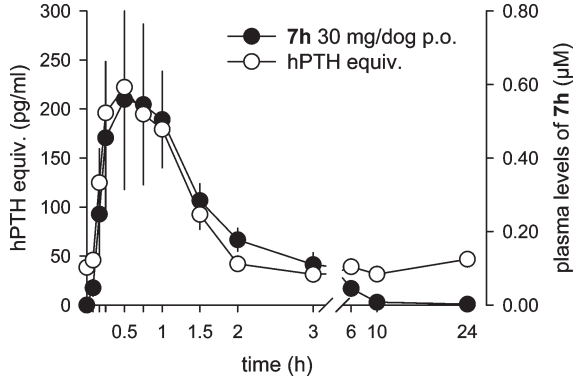


Figure 8. Plasma levels and bioactive parathyroid hormone (PTH, expressed as human PTH equivalents) after oral administration of a microemulsion formulation of **7h** at a dose of 30 mg per dog.

ing contrast to the exposure curve obtained with the EtOH/corn oil formulation, a pronounced and early peak was obtained with **7h** in the microemulsion and C_{max} was at least 5-fold higher. Furthermore, one hour after oral administration, drug levels had already dropped to values below 20% of their maximum levels (Figure 6).

The same microemulsion formulation was used for a single dose pharmacodynamic (PD) experiment in rats. While a dose of 10 mg/kg of **7h** was too low to induce PTH release, a sharp PTH peak with a C_{max} of ca. 150 pg·equiv/mL resulted with the higher dose of 30 mg/kg (cf. Figure 7).

Compound **7h** was also tested in dogs. Similar to what was previously seen in rats not only PK but also PD (PTH release) showed a perfectly sharp peak (Figure 8). The PK and PD curves are almost super imposable. Baseline for PTH levels in the experiment was around 50 pg·equiv/mL and was reached again 2 h post dosing. However, after oral dosing of 30 mg per dog (ca. 3 mg/kg), exposure was considerably higher than that observed in rats at the same dose (data not shown).

In vitro, the most potent calcilytics of our series were those with a thiocarbonyl group (**11**, cf. Table 3). As an example, **11m** with subnanomolar potency was selected to be tested in vivo in rats and dogs. In the rat, a 10× lower dose (3 mg/kg) resulted in a peak similar in shape to that obtained with **7h** at 30 mg/kg reaching a PTH C_{max} of ca. 280 pg·equiv/mL (Figure 9).

The superior potency of **11m** was also seen in the dog in which a dose of 3 mg/dog (approximately 0.3 mg/kg) was

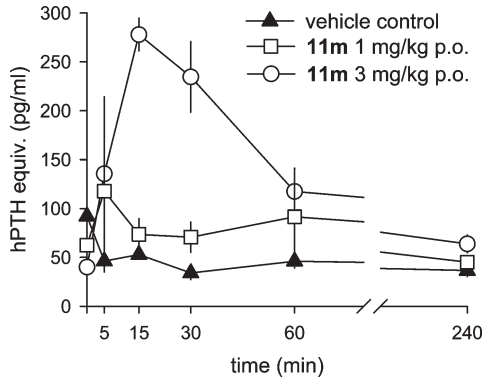


Figure 9. Plasma levels and bioactive parathyroid hormone (PTH, expressed as human PTH equivalents) after oral administration of a microemulsion formulation of **11m** at a dose of 1 and 3 mg/kg to rats.

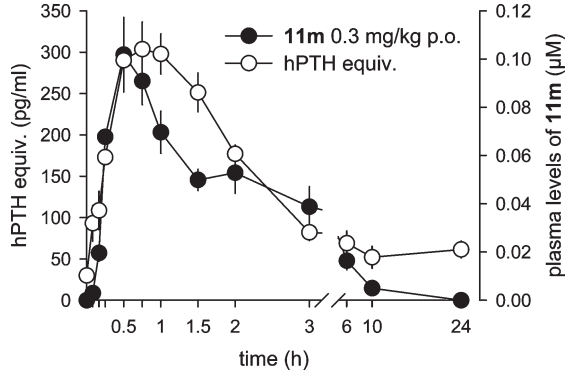


Figure 10. Plasma levels of PTH and of parent compound **11m** after oral administration of 3 mg/dog (ca. 0.3 mg/kg) in a microemulsion formulation.

sufficient for a PTH C_{max} of around 300 pg·equiv/mL (Figure 10). In both species, rat and dog, **11m** was thus 10 times more potent than **7h**.

In summary, a highly potent and novel series of calcilytics was discovered. Optimization by classical medicinal chemistry led to compound **11m**, which showed a sharp PTH release peak in rats and dogs provided that a microemulsion formulation is used. The maximum PTH levels achieved as well as the sharp profile suggest that compounds such as **11m** should have an anabolic effect on bone. Data from long-term in vivo studies of a derivative of the current lead series will be reported shortly elsewhere.

Experimental Procedures

Assay for Intracellular Free Calcium. Antagonism at the CaSR was determined by measuring the inhibition of intracellular calcium transients stimulated by extracellular calcium. CCL39 fibroblasts stably transfected with human CaSR were seeded at 40000 cells/well into 96-well Viewplates and incubated for 24 h. Medium was then removed and replaced with fresh medium containing 2 μM Fluo-3 AM (Molecular Probes, Leiden, The Netherlands). In routine experiments, cells were incubated at 37 °C, 5% CO_2 for 1 h. Afterward, plates were washed twice with mHBS and wells were refilled with 100 μL of mHBS containing the test compounds. Incubation was continued at room temperature for 15 min. To record changes of intracellular free calcium, plates were transferred to a fluorescence-imaging plate reader (FLIPR, Molecular Devices, Sunnyvale, CA). A baseline consisting in five measurements of 0.4 s

Table 6. Composition of Microemulsion Preconcentrates (%, w/w)

component	MEPC A	MEPC B
Cremophor RH40	45.0	58.1
corn oil gylcerides (LCTG)	36.0	
Labrafil M2125 CS		16.9
propylene glycol	9.0	8.3
EtOH	10.0	16.7

each (laser excitation 488 nm) was recorded. Cells were then stimulated with calcium (2.5 mM final), and fluorescence changes were recorded over a period of 3 min.

Compositions of Microemulsion Preconcentrates. The microemulsion preconcentrates were prepared as follows: After heating Cremophor RH40 to 65 °C with stirring, the other components were added at the weight ratios indicated in Table 6. This mixture was stirred for 1 h. The clear solution obtained (microemulsion preconcentrate; MEPC) was mixed with the test compound, and the resulting mixture was stirred at ambient temperature for 8−12 h. Complete dissolution in the microemulsion preconcentrate was assessed by crossed polarized light microscopy.

In Vivo Experiments. Measurement of Parathyroid Hormone (PTH) Release in Anesthetized Rats after Single iv Dosing. In male Wistar rats (300−350 g body weight), one of the carotid arteries (for rapid blood sampling) and one of the jugular veins (for iv drug administration) were cannulated under anesthesia. Blood samples were collected immediately before (0 min) and after iv bolus drug administration, and serum was separated from blood cells by a centrifugation step. Serum samples were stored for a maximum of 2 weeks until determination of PTH and compound levels.

Immunoreactive PTH in rat serum samples was determined using the Nichols Institute rat PTH (IRMA) kit (Immutopics Inc., San Clemente, CA).

Absorption Profile in Conscious Rats after Single po Dosing. Conscious rats (n = 4), permanently cannulated at the femoral artery, were treated with 3 mg/kg of **7h** by gavage, using a 1% solution in MEPC A diluted with deionized water to 2.5 mL final volume. Blood samples were taken at 0, 5, 15, 30, 60, 120, 180, 240, 360, 480, and 1440 min after administration.

Measurement of Parathyroid Hormone (PTH) Release in Rats after Single po Dosing. Three-month-old female Wistar rats were administered orally with the test compound or vehicle (microemulsion) alone. The microemulsions used were prepared starting with 2% w/w solution of the compounds in MEPC B, which were subsequently diluted with deionized water according to the doses applied (application volume 5 mL/kg). Animals were sacrificed by decapitation 5, 15, 30, 60, and 240 min after application. A group receiving vehicle alone before sacrifice served as baseline control. Trunk blood was taken at necropsy. EDTA plasma was collected for determination of PTH levels (rat PTH IRMA, Immutopics Inc., USA).

Measurement of Parathyroid Hormone (PTH) Release in Dogs after Single po Dosing. Fasted male beagle dogs (aged 2−9 years; n = 3 per group) weighing about 10 kg were dosed as follows: A solution of 30 mg of **7h** in 3.0 g MEPC A was diluted 10-fold with deionized water to a final volume of 30 mL, mixed well, and administered by gavage with a subsequent rinse with 15 mL of tap water. 3 mg of compound **11k** were dissolved in 0.5 g of MEPC B, diluted with deionized water to a final volume of 15 mL, mixed well, and administered by gavage with a subsequent rinse with 25 mL of tap water. Blood samples from conscious dogs were taken into EDTA-coated tubes at 0, 5, 10, 15, 30, 45, 60, 90, 120, 180, 360, 600, and 1440 min post administration. Plasma concentrations of parent compound were determined using a specific HPLC/MS-MS method, and levels of bioactive parathyroid hormone (hPTH) were determined with an intact PTH radioimmunoassay.

General. Reagents and solvents were used as purchased without further purification. Flash chromatography was carried out on a Flash Master II system with E. Merck Kieselgel 60 (230−400 mesh) silica gel or a Combi Flash Companion system using prepacked columns. Reactions were monitored either by TLC (E. Merck Silica Gel 60 F 254 thin layer plates) or by LC/MS (Agilent 1100 series with a G1946 D mass spectrometer using atmospheric pressure chemical ionization (APCI), which was also used for all MS data provided. The purity of all compounds for biological testing was determined by high performance liquid chromatography. Method A: Agilent 1100 HPLC with diode array detection in the range of λ = 210−250 nm, column: Waters XTerra MS C_{18} 50 mm × 4.6 mm Ø 3.5 μm pore size, flux: 4 mL/min containing 0.1 % TFA with a linear gradient going form 90% water/10% acetonitrile to 5% water/95% acetonitrile in 8.3 min. Method B: the same as method A but with a Waters XTerra Phenyl (50 mm × 2.1 mm Ø3.5 μm pore size) column and a flux of 2 mL/min. All compounds analyzed by these methods possessed purities equal to or greater than 95%, with the exception of **21a** (100% using method A and 94% method B).

Nuclear magnetic resonance spectra were recorded at 300 or 400 MHz using Varian Gemini or Mercury instruments. Chemical shifts are given in ppm.

5-Methoxy-2-nitro-benzaldehyde (3). A mixture of 5.0 g (29.9 mmol) 5-hydroxy-2-nitro-benzaldehyde, 2.05 mL (32.9 mmol) of iodomethane, and 4.14 g (29.9 mmol) of potassium carbonate in 50 mL of DMF was stirred for 12 h at rt. The resulting yellow suspension was taken up in water and ethyl acetate. The crude product was recrystallized from a mixture of ethyl acetate/petroleum ether to yield 4.57 g (84%) of compound **3** in the form of a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.49 (s, 1H), 8.16 (d, 1H), 7.33 (d, 1H), 7.15 (dd, 1H), 3.96 (s, 3H).

(4-Isopropyl-phenyl)-(5-methoxy-2-nitro-phenyl)-methanol (4). A suspension of 486 mg (20.0 mmol) of magnesium in 2 mL of THF was treated with 100 mg of 4-isopropyl-bromobenzene (in 0.5 mL of THF). The Grignard reaction was started by gentle heating and the rest of the bromide (diluted with 8 mL of THF) was added within 10 min (total amount 2.39 g; 12 mmol). After heating to reflux for $^1/_2$ h, the resulting mixture was added dropwise to a suspension of 1.81 g (10.0 mmol) of **3** in 30 mL of THF at −70 °C. After 2 h at this temperature, the reaction was quenched by the addition of satd ammonium chloride solution and the product was extracted with ethyl acetate. The yellow oily product was purified by flash-chromatography (hexanes/ethyl acetate). Yield: 1.65 g (55%) of **4** as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 (d, 1H), 7.35 (d, 1H), 7.25 (d, 2H), 7.18 (d, 2H), 6.89 (dd, 1H), 6.52 (broad s, 1H), 3.90 (s, 3H), 2.88 (hept, 1H), 2.71 (broad, OH), 1.22 (d, 6H). MS: 284 $(M - OH)^+$.

(2-Amino-5-methoxy-phenyl)-(4-isopropyl-phenyl)-methanone (5). The alcohol **4** (1.6 g; 5.31 mmol) prepared in the step above was dissolved in 5 mL of acetone, cooled to 0 °C, and treated dropwise with 3 mL of a 2.6 M solution of Jones reagent.[25] The mixture turned immediately dark. After stirring for 1 h, the chromium salts were separated and washed several times with acetone. After stripping off the acetone in vacuo, the filtrate was extracted with water/ethyl acetate. Evaporation of the organic layers afforded 1.47 g (92%) of crude (4-isopropyl-phenyl)-(5-methoxy-2-nitro-phenyl)-methanone. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (d, 1H), 7.69 (d, 2H), 7.29 (d, 2H), 7.07 (dd, 1H), 6.87 (d, 1H), 3.92 (s, 3H), 2.96 (hept, 1H), 1.26 (d, 6H).

The nitro group of the material obtained above was catalytically hydrogenated at atmospheric pressure and rt with Ra−Ni (0.5 g) in 30 mL of MeOH/THF 1:1. Some over-reduction to the amino alcohol was observed. This material was separated by chromatography (hexanes/ethyl acetate) to yield 550 mg (42%) of **5**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.63 (d, 2H), 7.31 (d, 2H), 7.01 (d, 1H), 6.98 (dd, 1H), 6.72 (d, 1H), 3.68 (s, 3H), 2.98 (hept, 1H), 1.29 (d, 6H). MS: 270 $(M + 1)^+$.

(2-Isopropyl-amino-5-methoxy-phenyl)-(4-isopropyl-phenyl)-methanone (6). A mixture of the amine **5** (500 mg; 1.86 mmol) and 308 mg (2.23 mmol) of potassium carbonate in 33 mL of 2-iodopropane was stirred for two days at 110 °C. The mixture

was distributed between ethyl acetate and water, the layers were separated, and the organics concentrated in vacuo. Flash-chromatography (ethyl acetate/hexanes) yielded 530 mg of a yellow oil, which was a mixture of the desired monoalkylated **6** and some dialkylated material (8%). This mixture was used for the next cyclization step without further purification.

1-Isopropyl-4-(4-isopropyl-phenyl)-6-methoxy-1H-quinazolin-2-one (2a). A solution of 250 mg (0.80 mmol) of **6** in 5 mL of glacial acetic acid was treated at rt with 81 mg (1.2 mmol) of sodium cyanate. Workup after stirring overnight afforded a yellow oil, which was purified by chromatography (petroleum ether/ethyl acetate) to give a yellow solid. After recrystallization, 150 mg (56%) of **2a** were obtained; mp 159−162 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (d, 2H), 7.54 (d, 1H), 7.29−7.40 (m, 4H), 5.21 (broad, 1H), 3.78 (s, 3H), 3.00 (broad hept, 1H), 1.69 (d, 6H), 1.30 (d, 6H). MS: 337 $(M + 1)^+$.

4-(4-*tert*-Butyl-phenyl)-1-isopropyl-6-methoxy-1*H*-quinazolin-2-one (2f). 2f was prepared according to the method described by Houlihan et al.[14] A suspension of 200 mg (0.96 mmol) of 1-isopropyl-1-(4-methoxy-phenyl)-urea, 192 μL (1.15 mmol) of 4-*tert*-butylbenzaldehyde, and 32 μL (0.49 mmol) of methanesulfonic acid in 1 mL of diglyme was heated at 130 °C overnight. The reaction mixture was poured into water and extracted with ethyl acetate. The crude product was purified by preparative HPLC to yield 163 mg (0.46 mmol, 48%) of the intermediate 4-(4-*tert*-butyl-phenyl)-1-isopropyl-6-methoxy-3,4-dihydro-1*H*-quinazolin-2-one.

The whole amount of this intermediate was dissolved in 3 mL of 1,4-dioxane and treated at 10 °C with a solution of 88 mg (0.56 mmol) of $KMnO_4$ in 2 mL of water followed by the addition of 37 μL (0.48 mmol) of 36% formalin solution. After 20 min, the reaction mixture was filtered and the filtrate was evaporated. The residue was purified by chromatography (ethyl acetate/hexanes) to yield 84 mg (52%) of **2f**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.71 (d, 2H), 7.54 (d, 1H), 7.35−7.31 (m, 4H), 5.21 (hept, 1H), 3.79 (s, 3H), 1.69 (d, 6H), 1.38 (s, 9H). MS: 351 $(M + 1)^+$.

6-Hydroxy-1-isopropyl-4-(4-isopropyl-phenyl)-1*H*-quinazolin-2-one (7a). To a solution of 5.0 g (14.9 mmol) of **2a** in 20 mL of CH_2Cl_2, 29.8 mL (29.8 mmol) of 1 M solution of BBr_3 in CH_2Cl_2 were added dropwise, causing a slight exotherm. After stirring for 3 h at rt, the reaction mixture was poured into water and extracted with CH_2Cl_2. Crystallization from diethyl ether afforded 3.66 g (76%) of **7a**. ^{1}H NMR (CD_3OD, 300 MHz): δ 8.00 (d, 1H), 7.76 (d, 2H), 7.74 (dd, 1H), 7.65 (d, 2H), 7.28 (d, 1H), 5.34 (m, 1H), 3.12 (hept, 1H), 1.72 (d, 6H), 1.36 (d, 6H). MS: 323 $(M + 1)^+$.

6-Allyloxy-1-isopropyl-4-(4-isopropyl-phenyl)-1*H*-quinazolin-2-one (7f). To a solution of 3.66 g (11.4 mmol) of **7a** in 20 mL of DMEU, 5.57 g (17.1 mmol) of cesium carbonate were added, followed by 2.76 g (22.8 mmol) of allyl bromide. After stirring for 18 h at rt, water was added and the product was extracted with diethyl ether. Chromatography (hexanes/ethyl acetate) afforded 2.96 g (72%) of **7f**. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.68 (dt, 2H), 7.52 (d, 1H), 7.38−7.32 (m, 4H), 6.00 (ddt, 1H), 5.37 (dq, 1H), 5.31 (dq, 1H), 5.20 (hept, 1H), 4.50 (dt, 2H), 3.00 (hept, 1H), 1.69 (d, 6H), 1.31 (d, 6H). MS: 363 $(M + 1)^+$.

(2-Benzyl-amino-5-propargyloxy-phenyl)-(4-isopropyl-phenyl)-methanone (9a). A mixture of 1.00 g (3.41 mmol) (2-amino-5-proparyloxy-phenyl)-(4-isopropyl-phenyl)-methanone (**8**), 0.49 mL (4.10 mmol) of benzyl bromide, and 0.70 mL (4.09 mmol) of Huenig's base in 10 mL of 1,4-dioxane was stirred for 7 h at 100 °C. The crude product obtained after extraction with ethyl acetate was purified by flash chromatography (ethyl acetate/hexanes) to yield 1.23 g (88%) of **9a** in the form of a yellow oil. ^{1}H NMR (300 MHz, CD_3OD): δ 7.58 (d, 2H), 7.39−7.31 (m, 6H), 7.27−7.22 (m, 1H), 7.13−7.08 (m, 2H), 6.78 (d, 1H), 4.53 (s, 2H), 4.48 (s, 2H), 3.00 (hept, 1H), 2.91 (s, 1H), 1.30 (d, 6H). MS: 384 $(M + 1)^+$.

1-Benzyl-4-(4-isopropyl-phenyl)-6-propargyloxy-1*H*-quinazolin-2-one (10a). A solution of 0.5 g (1.15 mmol) of **9a** and 78 mg (1.15 mmol) of sodium cyanate in 12 mL of glacial acetic acid was stirred for 4 h. After careful neutralization with 0.1 N NaOH solution, the reaction mixture was extracted with ethyl acetate. The crude product was purified by flash-chromatography (ethyl acetate/hexanes). After recrystallization, 240 mg (36%) of **10a** was obtained as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (d, 2H), 7.49 (s, 1H), 7.39 (d, 2H), 7.23−7.34 (m, 7H), 5.56 (s, 2H), 4.63 (s, 2H), 3.02 (hept, 1H), 2.53 (s, 1H), 1.32 (d, 6H). MS: 409 $(M + 1)^+$.

{2-[3-(2-Hydroxy-ethoxy)-benzylamino]-5-propargyloxy-phenyl}-(4-isopropyl-phenyl)-methanone (9m). A solution of 4.00 g (13.6 mmol) of the aniline **8** in 50 mL of dichloromethane was treated at rt with 2.43 g (14.3 mmol) of 3-(2-hydroxyethoxy)-benzaldehyde and 3.35 g (15.0 mmol) of sodium triacetoxyborohydride. After stirring overnight at rt, another 0.3 equiv of the aldehyde and the hydride was added to drive the reaction to completion. Workup was done after a further 8 h by pouring the orange reaction mixture into 100 mL of water and extraction with dichloromethane. The crude yellow oil was purified by chromatography (ethyl acetate/hexanes) to yield 3.6 g (60%) of {2-[3-(2-hydroxy-ethoxy)-benzylamino]-5-propargyloxy-phenyl}-(4-isopropyl-phenyl) methanone (**9m**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.58 (broad, OH), 7.59 (d, 2H), 7.29 (d, 2H), 7.26 (t, 1H), 7.20−7.22 (m, 1H), 7.08 (dd, 1H), 6.97 (d, 1H), 6.94 (s, 1H), 6.80 (d, 1H), 6.66 (d, 1H), 4.52 (d, 2H), 4.43 (s, 2H), 4.03−4.08 (m, 2H), 3.91−3.94 (m, 2H), 2.98 (hept, 1H), 2.48 (t, 1H), 1.31 (d, 6H).

1-[3-(2-Hydroxy-ethoxy)-benzyl]-4-(4-isopropyl-phenyl)-6-propargyloxy-1*H*-quinazolin-2-one (10m). A solution of 2.50 g (5.64 mmol) of **9m** and 748 mg (11.3 mmol) of sodium cyanate in 45 mL of glacial acetic acid was stirred for 1 h at rt. After concentration in vacuo, the residue was poured into 100 mL of water and extracted with ethyl acetate. The organic layers were washed with 1 M aqueous potassium carbonate solution and brine to give a yellow foam on concentration in vacuo. The crude product was triturated with hexane to yield 2.1 g (80%) of the target compound **10m**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, 2H), 7.50 (d, 1H), 7.39 (d, 2H), 7.32 (dd, 1H), 7.23−7.28 (m, 2H), 6.93 (d, 1H), 6.89 (s, 1H), 6.81 (dd, 1H), 5.52 (broad s, 2H), 4.64 (d, 2H), 4.04 (t, 2H), 3.92 (t, 2H), 3.02 (hept, 1H), 2.54 (t, 1H), 1.32 (d, 6H). MS: 469 $(M + 1)^+$.

1-[3-(2-Hydroxy-ethoxy)-benzyl]-4-(4-isopropyl-phenyl)-6-propargyloxy-1*H*-quinazolin-2-thione (11m). A solution of 2.10 g (4.73 mmol) **9m** in 20 mL of THF was treated with 700 μL (5.2 mmol) of benzoyl isothiocyanate. After stirring for 2 h at rt, 654 mg (4.73 mmol) of potassium carbonate and 20 mL of methanol were added. The reaction was complete after a further 2 h and worked up by quenching with 200 mL of a brine/water mixture. Extraction with dichloromethane and concentration of the organic layers afforded an orange crude product, which was purified by chromatography (ethyl acetate/hexanes), resulting in 1.66 g of **11m** in the form of an orange foam. The product was further purified by trituration with diethyl ether. Yield: 1.23 g (50%); mp 84−86 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.82 (d, 2H), 7.52 (m, 1H), 7.20−7.42 (m, 5H), 6.76−6.94 (m, 3H), 6.18 (broad s, 2 H), 4.66 (d, 2 H), 4.03 (t, 2H), 3.92 (t, 2H), 3.00 (hept, 1H), 2.56 (t, 1H), 1.30 (d, 6H). MS: 485 $(M + 1)^+$.

(2-Chloro-5-nitro-phenyl)-4-isopropoxy-phenyl)-methanone (12). A solution of 11.1 g (60 mmol) of 2-chloro-5-nitrobenzaldehyde in 140 mL of anhydrous THF was treated at −75 °C dropwise with 86 mL (61.9 mmol) of 0.72 M 4-isopropyl-phenylmagnesium bromide. After complete addition, the mixture was allowed to come to rt. Extractive workup with diluted HCl solution/ethyl acetate resulted in 20.0 g of a dark oil, which was purified by flash-chromatography (hexanes/ethyl acetate) to give 15.0 g (81%) of (2-chloro-5-nitro-phenyl)-(4-isopropyl-phenyl)-methanol in the form of a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.71 (d, 1H), 8.10 (dd, 1H), 7.49 (d, 1H), 7.31 (d, 2H), 7.22 (d, 2H), 6,17 (d, 1H), 2.90 (hept, 1H), 2.88 (d, OH), 1.24 (d, 6H).

A solution of 1.2 g (3.92 mmol) of the alcohol prepared in the previous step in 10 mL of acetone was treated at 0 °C with 1.8 mL (4.79 mmol) of 2.66 M Jones reagent. Stirring was continued overnight at rt. The acetone was removed in vacuo, followed by extractive workup with ethyl acetate/water to result in 1.09 g (92%) of **12** in the form of a yellow solid; mp 86−89 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.29 (dd, 1H), 8.23 (d, 1H), 7.73 (d, 2H), 7.67 (d, 1H), 7.36 (d, 2H), 3.00 (hept, 1H), 1.29 (d, 6H). MS: 274 [$(M + 1)^+ - 30$].

(2-Isopropylamino-5-nitro-phenyl)-(4-isopropyl-phenyl)-methanone (13). A mixture of 300 mg (0.95 mmol) of **12** in 15 mL of isopropylamine was heated to 65 °C for 10 h in a sealed tube. The excess of amine was stripped off in vacuo. Extraction of the residue with ethyl acetate/water gave a quantitative yield of **13** in the form of a yellow resin, which was used without purification in the next step. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.24 (broad d, NH), 8.54 (d, 1H), 8.23 (dd, 1H), 7.57 (d, 2H), 7.35 (d, 2H), 6.78 (d, 1H), 3.88 (8-line system, 1H), 3.00 (hept, 1H), 1.36 (d, 6H), 1.31 (d, 6H). MS: 327 $(M + 1)^+$.

1-Isopropyl-4-(4-isopropyl-phenyl)-6-nitro-1*H*-quinazolin-2-one (14). The crude benzophenone derivative **13** was taken up into 10 mL of benzene and treated slowly with 0.10 mL (1.2 mmol) of chlorosulfonyl isocyanate (diluted with 2 mL of benzene). After stirring 1 h at rt, extractive workup with ethyl acetate/water afforded the crude product, which was purified by chromatography (petroleum ether/ethyl acetate). Yield (after recrystallization from diethyl ether): 140 mg (40%) of **14** in the form of a slightly yellow solid; mp 208−209 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.79 (d, 1H), 8.53 (dd, 1H), 7.71 (d, 2H), 7.67 (d, 1H), 7.43 (d, 2H), 5.14 (hept, 1H), 3.03 (hept, 1H), 1.73 (d, 6H), 1.33 (d, 6H). MS: 352 $(M + 1)^+$.

6-Amino-1-isopropyl-4-(4-isopropyl-phenyl)-1*H*-quinazolin-2-one (15). Catalytic hydrogenation of a solution of 4.05 g (11.5 mmol) of **14** in 150 mL ethanol/50 mL THF over Raney nickel (1 g) at rt and normal pressure resulted in a quantitative yield of **15** as a red solid, which was used for further transformations without purification; mp 218−220 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.65 (d, 2H), 7.42 (d, 1H), 7.34 (d, 2H), 7.08−7.13 (m, 2H), 5.18 (broad hept, 1H), 3.67 (broad, NH_2), 2.99 (hept, 1H), 1.67 (d, 6H), 1.30 (d, 6H). MS: 322 $(M + 1)^+$.

6-Allylamino-1-isopropyl-4-(4-isopropyl-phenyl)-1*H*-quinazolin-2-one (16e). To a solution of 40 mg (0.124 mmol) of **15** and 50 μL (0.29 mmol) of *N*,*N*-diisopropylethyl amine (in 2 mL of dichloromethane), 16 μL (0.13 mmol) of trimethylchlorosilane were added dropwise at rt. After stirring for 1 h at 50 °C, 1 equiv of allyl bromide (11 μL; 0.13 mmol) was added. The reaction was kept for 6 h at that temperature. The crude product obtained after extractive workup with ethyl acetate/water was purified by flash chromatography (ethyl acetate/hexanes) to give 10 mg (33%) of **16e**; mp 209−211 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.67 (d, 2H), 7.43 (d, 1H), 7.33 (d, 2H), 7.07 (dd, 1H), 6.99 (d, 1H), 5.80−5.94 (m, 1H), 5.13−5.26 (m, 3H), 3.89 (broad s, NH), 3.72 (broad d, 2H), 2.98 (hept, 1H), 1.66 (d, 6H), 1.29 (d, 6H). MS: 362 $(M + 1)^+$.

6-Dipropargylamino-1-isopropyl-4-(4-isopropyl-phenyl)-1*H*-quinazolin-2-one (16h). A mixture of 200 mg (0.622 mmol) of the aniline **15** in 2 mL of DMF and 260 mg (1.87 mmol) of potassium carbonate was treated with 0.14 mL (1.57 mmol) of propargyl bromide. The mixture was heated in a sealed tube for 12 h at 90 °C. Extraction with ethyl acetate/water resulted in a dark oil, which was purified by chromatography (petroleum ether/ethyl acetate). Yield: 120 mg (48%) as a yellow foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7,75 (d, 2H), 7.56 (d, 1H), 7.41−7.47 (m, 2H), 7.36 (d, 2H), 5.21 (broad hept, 1H), 4.06 (d, 4H), 3.00 (hept, 1H), 2.28 (broad t, 2H), 1.69 (d, 6H), 1.31 (d, 6H). MS: 398 $(M + 1)^+$.

(2-Allyl-6-amino-3-hydroxy-phenyl)-(4-isopropyl-phenyl)-methanone (18a). A mixture of 10.0 g (34.0 mmol) of **17** (prepared analogously to **8**), 100 mL of DMEU, and 50 mL of water was heated in an autoclave for 48 h at a interior temperature of 165 °C. Water was evaporated in vacuo. The remaining DMEU solution was put on a reversed phase C_{18} column, and the DMEU was washed down with water. The eluent was changed to MeOH to collect the crude product, which was purified by chromatography (hexanes/ethyl acetate) to yield 6.87 g (69%) of **18a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.79 (d, 2H), 7.30 (d, 2H), 6.81 (d, 1H), 6.60 (d, 1H), 5.80 (ddt, 1H), 5.03 (dq, 1H), 5.01 (dq, 1H), 3.16 (dt, 2H), 2.97 (hept, 1H), 1.28 (d, 6H). MS: 296 $(M + 1)^+$.

On smaller scale, heating **17** in a DMEU water mixture by microwave radiation to 180 °C for 30 min afforded **18a** readily.

(2-Allyl-6-benzylamino-3-hydroxy-phenyl)-(4-isopropyl-phenyl)-methanone (19a). To a solution of 500 mg (1.69 mmol) of **18a** in 3 mL of CH_2Cl_2, 171 μL (1.69 mmol) of benzaldehyde, 148 mg (2.37 mmol) of $NaCNBH_3$, and 96 μL (1.69 mmol) of glacial acetic acid were added. After stirring at rt for 150 min, 1 M aqueous HCl was added to quench the excess of hydride. The mixture was made basic by the addition of 1 M NaOH followed by extraction with CH_2Cl_2. Chromatography (hexanes/ethyl acetate) afforded 472 mg (72%) of **19a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.78 (d, 2H), 7.31 (d, 2H), 7.28−7.16 (m, 5H), 6.82 (d, 1H), 6.56 (d, 1H), 5.79 (ddt, 1H), 5.02 (dd, 1H), 5.01 (dd, 1H), 4.22 (s, 2H), 3.17 (d, 2H), 2.99 (hept, 1H), 1.30 (d, 6H). MS: 386 $(M + 1)^+$.

5-Allyl-1-benzyl-6-hydroxy-4-(4-isopropyl-phenyl)-1*H*-quinazolin-2-one (20a). A solution of 53.8 mg (140 μmol) of **19a** and 9.1 mg (140 μmol) of sodium cyanate in 0.8 mL of glacial acetic acid was stirred at rt for 18 h. Saturated sodium bicarbonate solution was added, and the product was extracted with CH_2Cl_2 to yield 42 mg (73%) of **20a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.48 (d, 2H), 7.33−7.24 (m, 8H), 7.14 (d, 1H), 5.65 (ddt, 1H), 5.52 (s, 2H), 5.10 (dd, 1H), 4.95 (dd, 1H), 3.20 (d, 2H), 2.97 (hept, 1H), 1.28 (d, 6H). MS: 411 $(M + 1)^+$.

1-Benzyl-6-hydroxy-4-(4-isopropyl-phenyl)-5-propyl-1*H*-quinazolin-2-one (20b). A solution of 200 mg (519 μmol) of **19a** in 6 mL of methanol was hydrogenated in the presence of Raney nickel during 2 h at normal pressure and rt. The catalyst was filtered off, and the filtrate was evaporated to yield 183.5 mg (91%) of **19b**. As described above for **20a**, **19b** was cyclized with NaOCN to afford **20b** in 87% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.58 (s, 1H), 7.41−7.16 (m, 11H), 5.43 (s, 2H), 2.99 (hept, 1H), 2.19 (m, 2H), 1.25 (d, 6H), 1.18 (m, 2H), 0.36 (t, 3H). MS: 413 $(M + 1)^+$.

(6-Amino-2-cyclopropylmethyl-3-hydroxy-phenyl)-(4-isopropyl-phenyl)methanone (18c). A diazomethane solution was freshly prepared by adding 515 mg (5 mmol) of *N*-nitroso-*N*-methylurea in small portions to a stirred mixture of 50 mL of diethyl ether and 17.5 mL of 40% aq potassium hydroxide solution at 5 °C. Ten minutes after the last addition, the organic layer was separated off and dried over solid potassium hydroxide. This diazomethane solution was added to a concentrated solution of 1.00 g (3.39 mmol) of **18a** in diethyl ether containing 152 mg (0.68 mmol) of $Pd(OAc)_2$. After stirring for 16 h at rt, the reaction mixture was filtered, evaporated to dryness, and purified by chromatography (hexanes/ethyl acetate) to yield 590 mg (56%) of **18c**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.78 (d, 2H), 7.29 (d, 2H), 6.77 (d, 1H), 6.55 (d, 1H), 4.70 (s, 1H), 3.40 (broad, 2H), 2.96 (hept, 1H), 2.35 (d, 2H), 1.27 (d, 6H), 0.88 (m, 1H), 0.38 (m, 2H), 0.09 (m, 2H). MS: 310 $(M + 1)^+$.

1-Benzyl-5-cyclopropylmethyl-6-hydroxy-4-(4-isopropyl-phenyl)-1*H*-quinazolin-2-one (20c). 20c was prepared from **18c** by reductive amination followed by treatment with NaOCN as described for **19a** and **20a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.00 (broad 1H), 7.47 (d, 2H), 7.29−7.22 (m, 8H), 7.01 (d, 1H), 5.49 (s, 2H), 2.96 (hept, 1H), 2.36 (d, 2H), 1.27 (d, 6H), 0.69 (m, 1H), 0.17 (m, 2H), −0.18 (m, 2H). MS: 425 $(M + 1)^+$.

***N*-{3-[4-(4-Isopropyl-pheny)-2-oxo-6-propargyloxy-2*H*-quinazolin-1-ylmethyl]-phenyl}-2-[4-(2-methoxy-ethyl)piperazin-1-yl]-acetamide (21b).** A suspension of 9.00 g (19.8 mmol) of 4-(4-isopropyl-phenyl)-1-(3-nitro-benzyl-6-propargyloxy-1*H*-quinazo-

lin-2-one (prepared analogously to compound **10a**) and 10 g (180 mmol) of iron powder in 180 mL of glacial acetic acid was stirred at 45 °C for 30 h. After 6 and 15 h, additional batches of 10 g of iron powder were added. The mixture was filtered through Hyflo and distributed between water and dichloromethane. The crude product was obtained as a yellow foam, which was purified by chromatography (ethyl acetate/petroleum ether) to yield 5.05 g (61%) of 1-(3-amino-benzyl)-4-(4-isopropyl-phenyl)-6-prop-2-ynyloxy-1*H*-quinazolin-2-one. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.75 (d, 2H), 7.48 (s, 1H), 7.39 (d, 2H), 7.30 (s, 2H), 7.11 (t, 1H), 6.73 (d, 1H), 6.62 (s, 1H), 6.57 (d, 1H), 5.46 (broad s, 2H), 4.64 (d, 2H), 3.65 (broad s, NH_2), 3.02 (hept, 1H), 2.54 (t, 1H), 1.32 (d, 6H).

The aniline (13.60 g; 32.0 mmol) obtained above was dissolved in 200 mL of dichloromethane and 5.4 mL (38.5 mmol) of triethylamine and was treated at −5 °C dropwise with 2.74 mL (35.2 mmol) of chloroacetyl chloride. After completed addition, the cooling bath was removed and the suspension allowed to come to rt. Workup was done after stirring for 2 h at rt by the addition of water and additional dichloromethane. A quantitative crude yield (16.0 g) of 2-chloro-*N*-{3-[4-(4-isopropyl-phenyl)-2-oxo-6-prop-2-ynyloxy-2*H*-quinazolin-1-ylmethyl]-phenyl}-acetamide was obtained in the form of dark-green crystals which were used without purification for the next step. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.34 (broad, NH), 7.72 (d, 2H), 7.60 (d, 1H), 7.49 (d, 1H), 7.46 (s, 1H), 7.37 (d, 2H), 7.27−7.35 (m, 3H), 7.12 (d, 1H), 5.53 (broad, 2H), 4.64 (d, 2H), 4.16 (s, 2H), 3.01 (hept, 1H), 2.55 (t, 1H), 1.32 (d, 6H).

A DMEU solution (25 mL) of 11.0 g (33.3 mmol) of the chloroacetyl derivative obtained above was treated with potassium carbonate (6.1 g; 44 mmol) and 1-(2-methoxyethyl)-piperazine (4.75 g; 33.0 mmol). The mixture was heated to 65 °C for 2 h. The yellow product was filtered off from the resulting suspension and washed with water. Furthermore, the filtrate was extracted with ethyl acetate, and the organic layers were concentrated in vacuo to give additional material. The combined crude material was purified by chromatography (dichloromethane/methanol). Yield: 14.0 g (98%) **21b**; mp 199−200 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.05 (broad, NH), 7.75 (d, 2H), 7.58 (s, 1H), 7.53 (d, 1H), 7.49 (d, 1H), 7.39 (d, 2H), 7.27−7.35 (m, 3H), 7.05 (d, 1H), 5.53 (broad s, 2H), 4.64 (s, 2H), 3.55 (broad t, 2H), 3.35 (s, 3H), 3.13 (s, 2H), 3.02 (hept, 1H), 2.75−2.56 (broad, 10H), 2.54 (t, 1H), 1.32 (d, 6H). MS: 608 $(M + 1)^+$.

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Supporting Information Available: Experimental information on synthetic preparation and analytical data of compounds **2b**−**2e**, **2g**−**2j**, **7b**−**7e**, **7g**−**7i**, **8**, **10b**−**10l**, **16a**−**16d**, **16f**, **16g**, **16i**, **21a**, and **21c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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