Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 1557–1560
Bioorganic & Medicinal Chemistry Letters

3*H*-Quinazolin-4-ones as a new calcilytic template for the potential treatment of osteoporosis

Irina Shcherbakova,* Manuel F. Balandrin, John Fox, Anjan Ghatak,✠ William L. Heaton and Rebecca L. Conklin

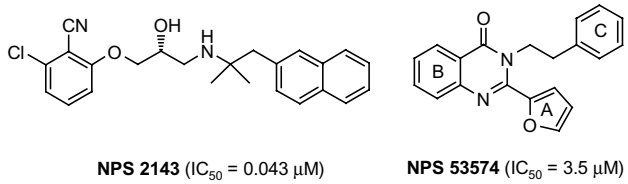
Drug Discovery, NPS Pharmaceuticals, Inc., 383 Colorow Drive, Salt Lake City, UT 84108, USA

Received 2 December 2004; revised 28 January 2005; accepted 31 January 2005

Abstract—Structure–activity relationship studies, focused on identification of the active pharmacophore fragments in a single high-throughput screening calcilytic hit, resulted in the discovery of potent calcium receptor antagonists, substituted 3*H*-quinazolin-4-ones.

Daily injections of parathyroid hormone (PTH) that induce transient increases in PTH levels stimulate the growth of new bone and might be a new anabolic therapy for osteoporosis.[1] An alternative therapeutic approach might involve stimulation of the secretion of endogenous PTH from the parathyroid glands that is regulated by the calcium receptor (CaR).[2,3] Compounds that block CaR activity (calcilytics) stimulate transient PTH release producing anabolic effects in bone and thus are implicated as a viable treatment for osteoporosis.[3,4] As demonstrated in animal studies, the first reported small molecule calcilytic, NPS 2143 (Fig. 1) induced the secretion of PTH and stimulated the growth of new bone.[4,5] Recently, a novel class of calcium receptor antagonists, N^1-arylsulfonyl-N^2-(1-(1-naphthyl)ethyl)-1,2-diamino-cyclohexanes have been discovered.[6] The ligand binding site model for the in vitro potent compound, Calhex 231 has been proposed,[7] although no in vivo effect has been reported for this pharmacophore type.

As part of an ongoing search for novel drug-like calcilytics with a good potential for optimization into potent, selective, nontoxic compounds with acceptable pharmacokinetic properties, we have identified a structurally new single hit, 3-phenethyl-2-furan-2-yl-3*H*-quinazolin-4-one, NPS 53574 (Fig. 1) via high-throughput screening. The biological activities of substituted 3*H*-quinazolin-4-ones are well documented; examples include sedative–hypnotic (methaqualone),[8] antitussive (chloroqualone),[9] and analgesic (diproqualone).[10] Herein, we describe a succinct structure–activity relationship (SAR) study around NPS 53574 that led to the discovery of novel potent CaR antagonists.[11,12]



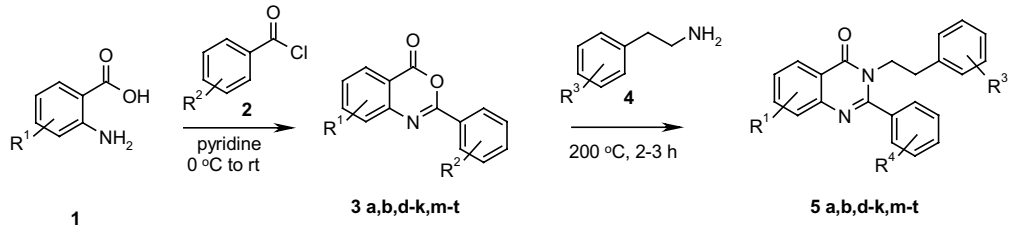
NPS 2143 (IC_{50} = 0.043 μM) **NPS 53574** (IC_{50} = 3.5 μM)

Figure 1.

We first compared the effect on calcilytic activity of replacing the 2-furan-2-yl function (ring A) in NPS 53574 with an unsubstituted and substituted phenyl ring. Evaluation of the substitution in the annelated benzene ring B and in the phenyl ring C of the 3-phenethyl substituent of 3*H*-quinazolin-4-ones targeted identification of the active pharmacophoric regions. The general method for synthesis of 2-aryl-substituted 3*H*-quinazolin-4-ones is the modification of literature methods[13,14] and shown in Scheme 1. The cyclization of substituted anthranilic acids **1** with aroyl chlorides **2** was accomplished in pyridine at room temperature to give benzoxazinones **3** in 60–90% yield. Heating

Keywords: Calcium receptor antagonists; Calcilytics; Osteoporosis; 3*H*-Quinazolin-4-ones.
* Corresponding author. Tel.: +1 801 583 4939; fax: +1 801 583 4961; e-mail: ishcherbakova@npsp.com
✠ Deceased.

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.01.078



Scheme 1. Compounds **3**, **5**: $R^2 = R^4$ if $R^2 \neq$ OAc or 2-OMe; R^4 = OH if R^2 = OAc or 2-OMe. For other substituents, see Tables 1 and 2.

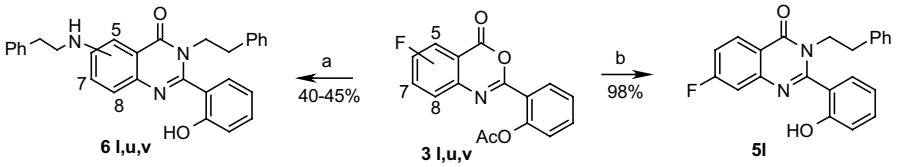
compounds **3** with a 10-fold excess of the amines **4** produced 3*H*-quinazolin-4-ones **5** in good to excellent yields, while the earlier method reported the formation of the mixture of quinazolinones and the ring-opened byproducts, bisamides.[14]

During the course of the latter reaction, undesired nucleophilic displacement of the fluorine substituent at position 7, 8, or 5 in benzoxazinones **3l**,**u**,**v** led to the amino-substituted quinazolinones **6l**,**u**,**v**, respectively, as the major products (Scheme 2), probably due to the reaction conditions (elevated temperature and the absence of solvent). The application of microwave irradiation resulted in the preparation of 7-fluoro-substituted quinazolinone **5l**, whereas 8- and 5-fluoro-substituted benzoxazinones **3u**,**v** still produced products of nucleophilic displacement **6u**,**v**.

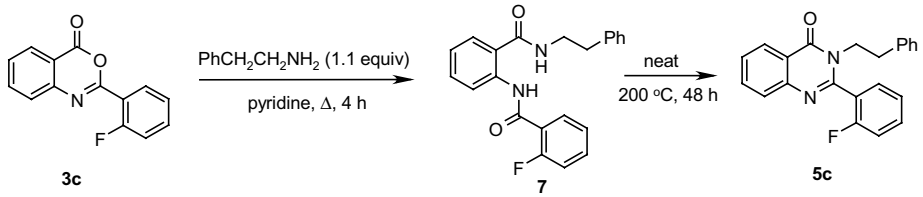
Similarly to the formation of the amino-substituted quinazolinones **6l**,**u**,**v**, fusion of 2-(2-fluoro-phenyl)-substituted benzoxazinone **3c** and phenethylamine (1.1 equiv) at 200 °C resulted in the dominant nucleophilic displacement of the fluorine substituent with the amino moiety. For preservation of the 2-(2-fluoro-phenyl) fragment, synthesis of the intermediate bisamide **7** was carried out in pyridine at 120 °C followed by cyclization to 2-(2-fluoro-phenyl)-3-phenethyl-3*H*-quinazolin-4-one **5c** on fusion (Scheme 3) similarly to the previously reported procedure.[13]

The 2-acetoxy group in the benzoxazinones **3** was cleaved under the reaction conditions to form the phenol moiety in quinazolinones **5**, even if a reduced excess of amines **4** (2-fold instead of 10-fold) was used as exemplified in Scheme 2. Surprisingly, regioselective demethylation was observed on interaction of 2-(methoxy-phenyl)-substituted benzoxazinones **3d**–**f** with phenethylamine: whereas a methoxy group at the *meta*- or *para*-position of the 2-phenyl ring remained unchanged, the *ortho*-methoxy group was cleaved yielding the hydroxy-substituted quinazolinone **5j** (Scheme 4). A similar regioselective demethylation of substituted 2-methoxybenzoic acids and their amides was recently reported as the first and so far only example of nucleophilic *O*-dealkylation in the presence of aliphatic amines.[15] The use of 1.1 equiv of phenethylamine in the reaction with benzoxazinone **3f** resulted in a complex mixture. The 2-(2-methoxy-phenyl)-substituted quinazolinone **5f** was obtained by methylation of the phenol **5j** (Scheme 4).

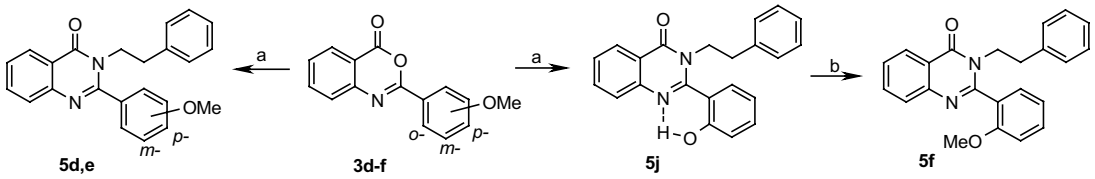
The quinazolinone compounds **5a**–**t** were evaluated in vitro for their calcilytic activity. Calcilytic activity was determined by a compound's ability to block, in a concentration-dependent manner, increases in the concentration of intracellular Ca^{2+} elicited by increases in extracellular Ca^{2+} in HEK 293 4.0–7 cells stably expressing the human CaR. Increases in intracellular Ca^{2+}, measured using fluo-3, a fluorescent calcium indicator



Scheme 2. Reagents and conditions: (a) $PhCH_2CH_2NH_2$ (2 equiv), 200 °C, 4 h; (b) $PhCH_2CH_2NH_2$ (2 equiv), DMF, microwave irradiation, 240 °C, 10 min. Compounds **3l**: 7-F; **3u**: 8-F; **3v**: 5-F; **6l**: 7-$NHCH_2CH_2Ph$; **6u**: 8-$NHCH_2CH_2Ph$; **6v**: 5-$NHCH_2CH_2Ph$.



Scheme 3.



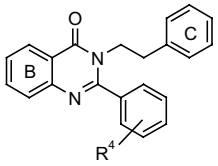
Scheme 4. Reagents and conditions: (a) $PhCH_2CH_2NH_2$ (2 equiv), 200 °C, 3 h; (b) MeI, K_2CO_3, THF, Δ, 24 h. Compounds **3**, **5**: **d** *m*-OMe; **e** *p*-OMe; **f** *o*-OMe.

(Biotium) were elicited by increasing extracellular Ca^{2+} from 1.0 to 1.3 mM. Fluorescence signals were measured as the peak height of the response and normalized to the response elicited by extracellular Ca^{2+} in the absence of test compound. All compounds were tested in duplicate at eight concentrations with the highest concentration being 30 μM. IC_{50} values of new compounds were normalized to that of a standard (internal reference) compound tested in parallel in each assay.

It was found that replacement of the 2-furan-2-yl fragment A in NPS 53574 (Fig. 1) with the phenyl ring decreased but did not erase calcilytic activity (**5a**, Table 1). Table 1 summarizes the SAR with respect to substitution in the 2-phenyl ring for quinazolinones **5a**–**j**. Introduction of the hydroxy substituents at the phenyl ring showed a trend toward increased calcilytic activity (**5g**,**i**), and the *ortho*-hydroxy-substituted compound **5j** demonstrated as much as a 50-fold potency increase when compared to the parent compound **5a**.

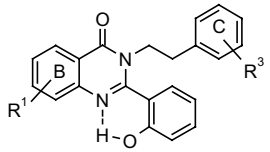
Thus, a single *ortho*-hydroxy substituent in the 2-phenyl ring of 3*H*-quinazolin-4-ones is beneficial to the calcilytic activity. The effect of such a substitution might be the result of an intramolecular hydrogen bond in a bioactive conformation as illustrated for 2-(2-hydroxy-phenyl)-3*H*-quinazolin-4-one **5j** in (Scheme 4). The effect of a single intramolecular hydrogen bond on increasing permeability is known to be significant and can easily amount to a factor of 10 or more.[16] The contribution of this effect could be favorable to the overall pharmacological profile of the optimized compounds.

Table 1. 2-Phenyl ring SAR



Compound **5**	R^4	IC_{50} (μM)
a	H	14
b	3-F	>30
c	2-F	>30
d	3-OMe	>30
e	4-OMe	>30
f	2-OMe	>30
g	3-OH	2.8
h	4-OH	>30
i	2,5-Di-OH	1.6
j	2-OH	0.3

Table 2. B and C ring SAR



Compound **5**	R^1	R^3	IC_{50} (μM)
k	8-Me	H	0.7
l	7-F	H	0.6
m	7-Cl	3-F	1.6
n	6-Cl	H	0.3
o	6-Me	3-F	0.52
p	H	3-Cl	0.8
q	5-Me	H	0.25
r	5-Me	3-F	0.25
s	6-F	H	0.21
t	6-F	3-F	0.19

Further optimization of the compound **5j** via mono- and di-substitution in the rings B and C improved potency modestly and yielded several compounds with attractive profiles (Table 2).

In vivo, the quinazolinones **5** with IC_{50} values <0.5 μM were very effective at transiently increasing plasma PTH levels following bolus intravenous injection in rats. Thus, compound **5j** (3 or 10 μmol/kg) or vehicle was administered by i.v. injection over about 15 s to normal conscious male Sprague–Dawley rats with chronic indwelling arterial and venous catheters. Arterial blood samples were collected immediately before, and at 1, 5, 10, and 30 min after the start of the injection for

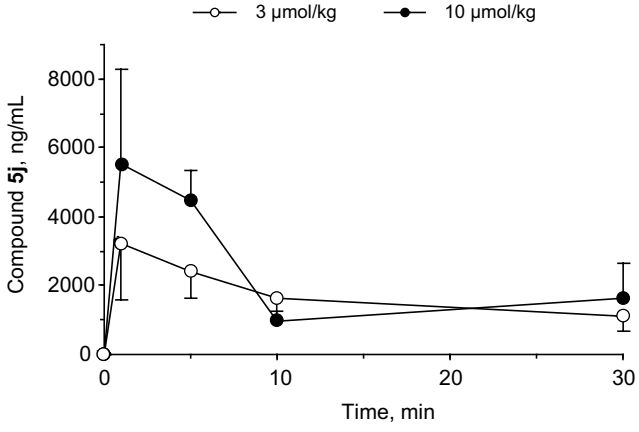


Figure 2. Plasma levels of compound **5j** after bolus i.v. injection in normal rats.

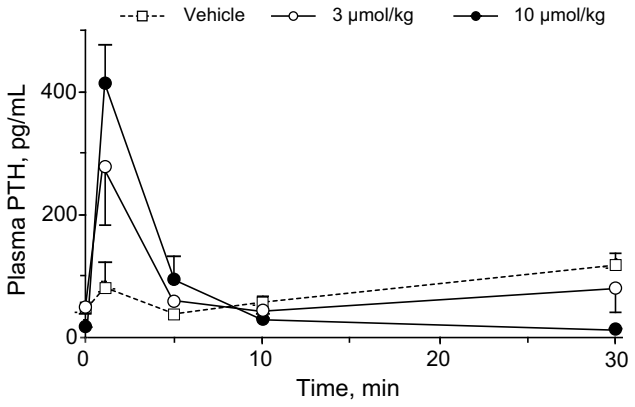


Figure 3. Effect of bolus i.v. injection of compound **5j** on plasma PTH levels in normal rats.

measurement of the levels of PTH, Ca^{2+}, and compound **5j** in plasma. PTH was measured using specific rat PTH(1-84) ELISA (Immutopics, San Clemente, CA) and compound **5j** was quantified by an LC–MS/MS method. Plasma levels of compound **5j** were maximal at 1 min after injection and declined rapidly during the next 10–30 min (Fig. 2). Injection of compound **5j** induced a rapid, but transient dose-related increase in plasma PTH levels that were maximal at 1 min after the injection. Plasma PTH levels had returned to pre-dose levels by 10 min (Fig. 3). There were no changes in plasma Ca^{2+} levels, probably because PTH levels were elevated for such a short time and/or because the study was only of 30 min duration. For example, when NPS 2143 (Fig. 1) was infused intravenously, plasma Ca^{2+} levels did not start to increase for 60 min.[5]

In summary, optimization of the substituted 3*H*-quinazolin-4-ones resulted in the generation of novel potent calcilytics, which stimulated PTH secretion in vivo. The series offers a new perspective for the development of clinically relevant calcium receptor antagonists.

References and notes

1. Fox, J. *Curr. Opin. Pharmacol.* **2002**, *2*, 338.
2. Fox, J.; Miller, M. A.; Stroup, G. B.; Nemeth, E. F.; Miller, S. C. *Bone* **1997**, *21*, 163.
3. Nemeth, E. F. *J. Mol. Endocrinol.* **2002**, *29*, 15.
4. Gowen, M.; Stroup, G. B.; Dodds, R. A.; James, I. E.; Votta, B. J.; Smith, B. R.; Bhatnagar, P. K.; Lago, A. M.; Callahan, J. F.; DelMar, E. G.; Miller, M. A.; Nemeth, E. F.; Fox, J. *J. Clin. Invest.* **2000**, *105*, 1595.
5. Nemeth, E. F.; DelMar, E. G.; Heaton, W. L.; Miller, M. A.; Lambert, L. D.; Conklin, R. L.; Gowen, M.; Gleason, J. G.; Bhatnagar, P. K.; Fox, J. *J. Pharmacol. Exp. Ther.* **2001**, *299*, 323.
6. Kessler, A.; Faure, H.; Roussanne, M. C.; Ferry, S.; Ruat, M.; Dauban, P.; Dodd, R. H. *ChemBioChem* **2004**, *5*, 1131.
7. Petrel, C.; Kessler, A.; Dauban, P.; Dodd, R. H.; Rognan, D.; Ruat, M. *J. Biol. Chem.* **2004**, *278*, 18990.
8. White, D. C.; Greenwood, T. D.; Downey, A. L.; Bloomquist, J. R.; Wolfe, J. F. *Bioorg. Med. Chem. Lett.* **2004**, *12*, 5711.
9. Buzas, A.; Hoffmann, C. *Bull. Soc. Chim. Fr.* **1959**, 1889.
10. Welch, W. M.; Ewing, F. E.; Huang, J.; Menniti, F. S.; Pagnozzi, M. J.; Kelly, K.; Seymour, P. A.; Guanowsky, V.; Guhan, S.; Guinn, M. R.; Critchett, D.; Lazzaro, J.; Ganong, A. H.; DeVries, K. M.; Staigers, T. L.; Chenard, B. L. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 177.
11. Shcherbakova, I.; Balandrin, M. F.; Fox, J.; Heaton, W. L.; Conklin, R. L.; Papac, D. I. WO 2004/031755 A3.
12. Shcherbakova, I.; Balandrin, M. F.; Fox, J.; Heaton, W. L.; Conklin, R. L.; Papac, D. I. *Drugs Future* **2004**, *29*(Suppl. A), 331.
13. Zentmyer, D. T.; Wagner, E. C. *J. Org. Chem.* **1949**, *14*, 967.
14. Wang, S.; Ryder, H.; Pretswell, I.; Depledge, P.; Milton, J.; Hancox, T. C.; Dale, I.; Dangerfield, P. C.; Charlton, P.; Faint, R.; Dodd, R.; Hassan, S. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 571.
15. Nishioka, H.; Nagasawa, M.; Yoshida, K. *Synthesis* **2000**, 243.
16. Gudmundsson, O. S.; Jois, S. D. S.; Vander Velde, D. G.; Siahaan, T. J.; Wang, B.; Bochardt, R. T. *J. Pept. Res.* **1999**, *53*, 383, Washington, DC 1997.