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Design, new synthesis, and calcilytic activity of substituted 3*H*-pyrimidin-4-ones

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Abstract—Design, new synthesis, structure–activity relationship studies and calcium receptor antagonist (calcilytic) properties of novel 3*H*-pyrimidin-4-ones are described.

A new anabolic therapy for osteoporosis that results in the growth of structurally normal new bone involves daily injections of parathyroid hormone (PTH).[1] An alternative therapeutic approach, which might overcome the systemic administration of costly peptides, is to stimulate 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 secretion of PTH.[4,5] Orally active small molecule calcilytics increase the level of circulating PTH and stimulate new bone formation in ovariectomized rats as was demonstrated for the first reported small molecule calcilytic, NPS 2143[4] (Fig. 1). This implicates calcilytics as a viable bone-building treatment for osteoporosis. There are a limited number of known chemotypes as calcium receptor antagonists, and those include N^1-arylsulfonyl-N^2-(1-(1-naphthyl)ethyl)-1,2-diamino-cyclohexanes,[6,7] 4-aryl-1*H*-quinazolin-2-ones,[8,9] 2-benzylpyrrolidine-substituted aryloxypropanols,[10] which are structurally similar to NPS 2143 (Fig. 1), and trisubstituted heteroaromatic derivatives.[11] Recently, we have described structure–activity relationship (SAR) studies around a new calcilytic hit, NPS 53574 that led to the discovery of novel potent CaR antagonists, 3*H*-quinazolin-4-ones **1** (Fig. 1).[12–14] Initial SAR of the quinazolinone series **1** rapidly revealed the key binding elements required to achieve a high level of potency and selectivity at CaR, and the 2-(2-hydroxy-phenyl) and 3-phenethyl substituents as essential for calcilytic activity.[12,14] Based on the SAR studies for compounds **1**, design of the 3*H*-pyrimidin-4-one template **2** (Fig. 1) was proposed as a series with a potentially improved

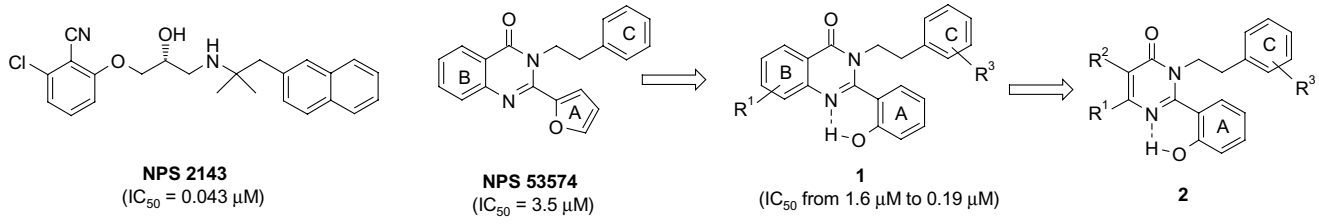

Figure 1.

Keywords: 3*H*-Pyrimidin-4-ones; Calcium receptor antagonists; Calcilytics; Osteoporosis.
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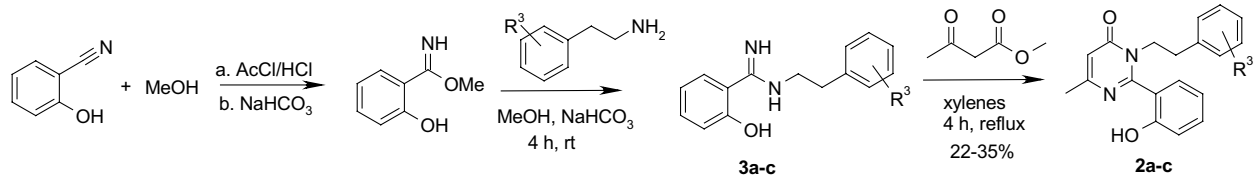
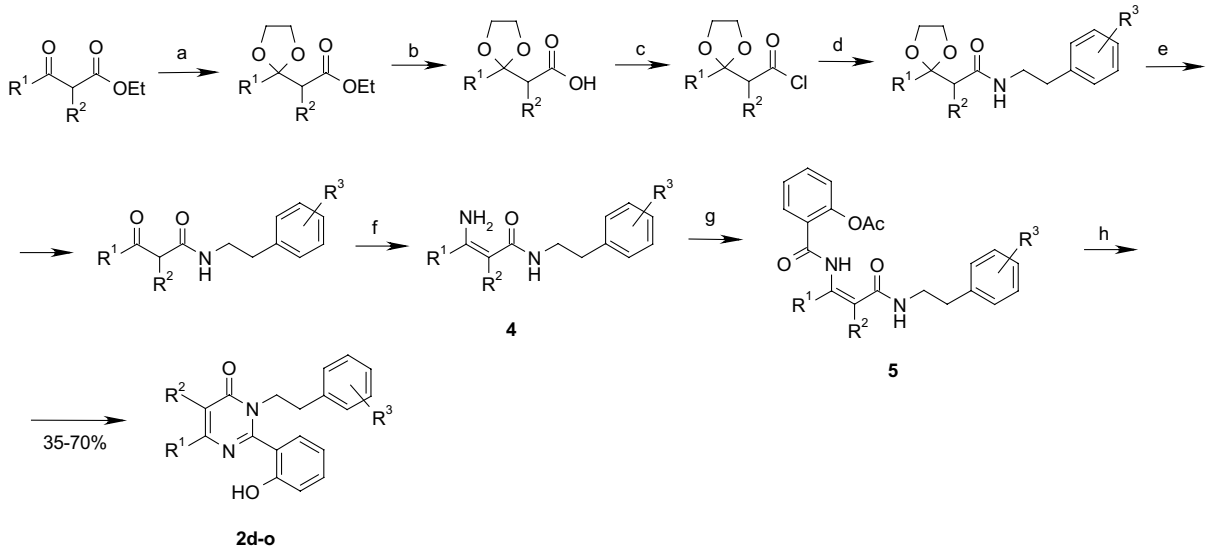
pharmacological profile. Pharmacological relevance of 3*H*-pyrimidin-4-ones is well recognized; examples include angiotensin II receptor antagonists,[15] PPAR agonists[16] and the atypical antipsychotic drug risperidone.[17]

We now report the design, new synthesis, and initial SAR studies for substituted 3*H*-pyrimidin-4-ones **2** that led to the generation of novel series of potent CaR antagonists.[18,19] During exploration of the SAR of the quinazolinone series **1**,[12] we found limited opportunity to modify substituents in rings B and C (Fig. 1) and retain or improve the activity profile. Equally important, the quinazolinone compounds **1** had relatively poor solubility (observed in handling but not quantitatively measured). Therefore, we turned to B ring modifications as we sought a means to optimize potency and solubility. Modifications in ring B were proposed by replacing a π-function of the annelated benzene ring in compounds **1** with one or two lipophilic fragments, the alkyl substituents that would result in pyrimidinone compounds **2** (Fig. 1). The synthesis of pyrimidinones **2a–c** unsubstituted at position 5 involved condensation of the amidines **3a–c** with 3-oxo-butyric acid methyl ester similar to the procedure described by Tice and Bryman[20] (Scheme 1).

However, 2-alkyl-3-oxo-butyric acid esters failed to produce 5,6-dialkyl substituted 3*H*-pyrimidin-4-ones **2** by this method. There are a limited number of routes to synthesize 2,3,5,6-substituted pyrimidinones, and those include (i) alkylation of 3*H*-pyrimidinones unsubstituted at N-3,[21,22] (ii) condensation of substituted amidines of type **3** (Scheme 1) with substituted malonyl chlorides,[23,24] and (iii) dehydration of an enediamide.[25] None of these approaches is general and each of them is limited by the nature of substituents in the pyrimidinone ring, and/or by the formation of byproducts, lengthy reaction time and low yields. Also, the target compounds **2** (Fig. 1) should contain a bulky functional group, the 2-(2-hydroxy-phenyl) substituent that limited the application of known methods. In search of a general approach to the target compounds **2**, we developed a novel synthesis of 2,3,5,6-substituted 3*H*-pyrimidin-4-ones via the key intermediates, eneaminoamides **4**[18] (Scheme 2). The subsequent acylation of compounds **4** with acetyl salicyl chloride produced the enediamides **5**. We optimized the conditions of cyclization of the enediamides **5**, and the final pyrimidinones **2d–o** were formed in moderate to good yields (Scheme 1), whereas the previously described procedure did not exceed a 12% yield.[25] Investigation on the scope of our pyrimidinone synthesis methodology, including introduction of sterically hindered substituents at N-3 and/or at position 5 of the heterocyclic ring, will be reported elsewhere. While our patent on synthesis of 2,3,5,6-substituted 3*H*-pyrimidin-4-ones was pending,[18] a practical synthesis of 3-substituted 3*H*-pyrimidinones via intermediate



Scheme 1. Compounds **2** and **3**: (a) R^3 = H; (b) R^3 = 2-F; (c) R^3 = 3-F.



Scheme 2. Reagents and conditions: (a) $HOCH_2CH_2OH$ (1.05 equiv), *p*-TosOH·H_2O (cat), anhydrous toluene, reflux; (b) KOH, dioxane–H_2O, 35 °C, 12 h; (c) oxalyl chloride (2.3 equiv) in CH_2Cl_2, 0 °C; 2 h, rt; (d) $(R^3)C_6H_4CH_2CH_2NH_2$, pyridine, 0 °C; rt, 12 h; (e) acetone–H_2O, *p*-TosOH·H_2O (cat), 95 °C, 3 h; (f) Et_2O, NH_3, $AlCl_3$ (anhydrous, 1 equiv); (g) 2-$AcOC_6H_4COCl$ (1 equiv), pyridine, reflux, 4 h; (h) EtOH–H_2O, KOH (1.5 equiv), reflux, 12 h. Compounds **2d–o**: for R^1, R^2, and R^3, see Table 1.

enamide esters, which are structurally similar to enediamides **5**, was published.[26] This method, however, fails to produce 3*H*-pyrimidin-4-ones with bulky alkyl substituents, such as isopropyl or *tert*-butyl, at N-3 and/or at positions 5.

Similarly to quinazolinones **1**,[14] the pyrimidinone compounds **2a**–**o** were evaluated in vitro for their calcilytic activity.[27]

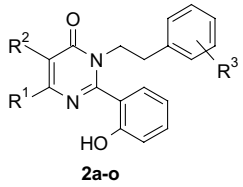
The data in Table 1 summarize an initial SAR around the 5,6-substitution in the compounds **2a**–**o**. The pyrimidinones **2a**–**c** unsubstituted at position 5 were 10-fold less potent than the parent quinazolinones **1**.[14]

5,6-Dimethyl substituted compounds **2d**,**e** showed markedly increased activity in comparison with 5-unsubstituted pyrimidinones **2a**–**c**. It is apparent that the alkyl substituent at position 5 of the pyrimidinone ring in compounds **2** is particularly important for calcilytic activity in this series. Introduction of the trifluoromethyl group at position 6 (compound **2f**) resulted in reduced potency. Thus, an additional electron-withdrawing effect at position 6 at the pyrimidinone ring was not beneficial for calcilytic activity. Starting with the increased potency of 5-ethyl-6-methyl compounds **2g**,**h** (Table 1), the alkyl substitution at position 5 was arbitrarily selected for exploration of structural modifications in the pyrimidinone ring. It was found that introduction of a propyl group at C-5 decreased potency slightly (compound **2i**) comparing to the 5-ethyl derivative **2g**, whereas an isopropyl group at C-5 and 3-fluoro substituent at 3-phenethyl fragment (compound **2j**) offered the most attractive package of potency and potential metabolic stability of fragments other than the 2-(2-hydroxy-phenyl) group. Cycloalkyl substitution involving C-5 and C-6 was well tolerated but did not lead to a significant potency enhancement (compounds **2l**–**o**). Also, in the pyrimidinone series **2**, fluoro substitution in the 3-phenethyl fragment did not influence potency to the same extent as in the quinazolinone series **1**.[12,14] Overall, the pyrimidinone compounds **2d**–**o** demonstrated a noticeable potency improvement in comparison with the parent series, quinazolinone compounds **1**[14] (Fig. 1). In addition, the pyrimidinones **2a**–**o** possessed good aqueous solubility (>10 μmol/L) in the physiological pH range. This might be due to the amphoteric nature of the 2-(2-hydroxy-phenyl) substituted pyrimidinones **2** that involve contributions to aqueous solubility from the ionizable OH group at pH >7, and from the protonated basic N-1 atom at pH <7.

In vivo, the pyrimidinones **2** with IC_{50} values <0.2 μM were more effective at lower doses than the quinazolinone calcilytics **1**[12,14] at transiently increasing plasma PTH levels following bolus iv injection in rats. Compound **2h** (1 or 3 μmol/kg) or vehicle was administered by intravenous 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 measurement of the levels of PTH and Ca^{2+} in plasma. PTH was measured using a specific rat PTH(1-84) ELISA (Immutopics, San Clemente, CA). Injection of compound **2h** 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. 2). Similarly to in vivo effect of the quinazolinone calcilytics,[14] 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.

In summary, the 3*H*-pyrimidin-4-ones represent a new and highly potent class of calcium receptor antagonists with an attractive pharmacological profile. Optimization of this calcilytic series to orally bioavailable compounds with balanced pharmacokinetic and pharmacodynamic properties is in progress.

Table 1. In vitro calcilytic activity of substituted 3*H*-pyrimidin-4-ones



2a-o

Compds	R^1	R^2	R^3	IC_{50}, μM
a	Me	H	H	1.9
b	Me	H	2-F	1.6
c	Me	H	3-F	1.1
d	Me	Me	H	0.2
e	Me	Me	3-F	0.17
f	CF_3	Me	H	0.8
g	Me	Et	H	0.1
h	Me	Et	2-F	0.097
i	Me	Pr	3-F	0.14
j	Me	Pr^i	3-F	0.095
k	Me	Cyclopropyl	3-F	0.12
l	–$(CH_2)_4$–		H	0.14
m	–$(CH_2)_4$–		3-F	0.16
n	–$(CH_2)_3$–		H	0.2
o	–$(CH_2)_3$–		3-F	0.2

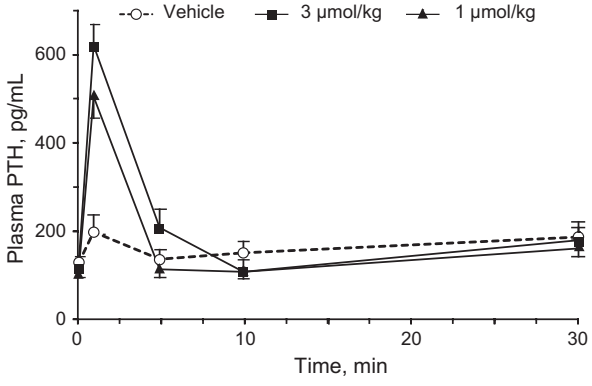


Figure 2. Effect of bolus iv injection of compound **2h** on plasma PTH levels in normal rats.

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27. 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 (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.