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# Synthesis and in vitro evaluation of derivatives of the $\beta_1$-adrenergic receptor antagonist HX-CH 44

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ABSTRACT

Isopropyl- and fluoroisopropyl-amino derivatives of the $\beta_1$-adrenergic receptor antagonist 2-[4-[3-(*tert*-butyl-amino)-2-hydroxypropoxy]phenyl]-3-methyl-6-methoxy-4(*3H*)-quinazolinone ((±)HX-CH 44) were synthesized, including a concise and efficient preparation of the core, 2-(4-hydroxyphenyl)-6-methoxy-3-methylquinazolin-4(*3H*)-one. In vitro binding assays showed that the fluorinated analog was selective towards $\beta_1$-adrenergic receptors over $\beta_2$-adrenergic and 5-$HT_{1A}$ receptors. An X-ray crystallographic characterization of the fluorinated analog is also reported.

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Recent crystal structures of the $\beta_1$- and $\beta_2$-adrenergic receptors[1–4] represent a major breakthrough in medicinal chemistry as their elucidation has significantly expanded opportunities for drug discovery targeting these therapeutically important G-protein-coupled receptors. Imaging of β-adrenergic receptors (β-ARs) in the heart or brain with radiolabeled β-adrenergic antagonists or agonists (β-blockers) using positron emission tomography (PET) is a longstanding goal of several laboratories,[5–7] including ours.[8,9] The most common methodology for such radiosyntheses involves the introduction of positron-emitting isotopes, $^{11}C$ (half-life = 20.4 min) or $^{18}F$ (half-life = 109.7 min), into the isopropylamine side-chain of the 1-isopropylamino-3-phenoxy-2-propanol moiety, a characteristic feature of several β-blockers. The use of [$^{11}C$]-CGP 12177[10–12] and its *N*-isopropyl analog [$^{11}C$]-CGP 12388,[13,14] are established for clinical heart imaging studies (Fig. 1), and are prepared using the technically demanding synthons [$^{11}C$]-phosgene[10] and [$^{11}C$]-acetone,[14] respectively. Neither compound is subtype-selective, and their widespread use has been restricted as the production of carbon-11 labeled radiotracers requires an on-site cyclotron. Our laboratory recently reported a facile 2-step procedure for the stereoselective syntheses of $^{18}F$-labeled β-blockers bearing a fluoroisopropylamino moiety[9] which is a common feature of several radiotracers for imaging β-ARs in vivo. This radiosynthetic method is being applied to discover new PET imaging agents for β-ARs.

Despite extensive efforts, there is still no radiopharmaceutical available for imaging $\beta_1$-ARs with PET.[6] A lead candidate, 2-[4-[3-(*tert*-butylamino)-2-hydroxypropoxy]phenyl]-3-methyl-6-methoxy-4(*3H*)-quinazolinone ((±)HX-CH 44), was selected for derivatization in the present study. This compound has been extensively characterized pharmacologically, both in vitro and in vivo.[15,16] Although the original report of (±)HX-CH 44 demonstrated that this drug is highly potent and selective (low nM affinity towards $\beta_1$ receptors in vitro with $\beta_1/\beta_2$ selectivity ratio of 800),[15] a subsequent study by the same group showed that these values are highly dependent on the assay and the $\beta_1$ affinity can fluctuate wildly (from 77 nM to 16 μM) with speculation of multiple binding sites in rodent heart membranes.[16] Racemic HX-CH 44 was previously radiolabeled with carbon-11 (Fig. 1) and evaluated for cardiovascular imaging in animal models.[17] Although [$^{11}C$]-(±)HX-CH 44 displayed minimal specific binding to myocardial $\beta_1$-ARs in the previous work, development of $^{18}F$-labeled derivatives of this compound may prove to yield promising radiotracers as $^{18}F$-labeling at the *N*-fluoroisopropyl moiety in this class of compounds has shown improved signal to noise compared with the respective $^{11}C$-labeled analogs.[6,7] In addition to the possibility of providing a better match of the pharmacokinetic profile to the lifetime of the radioisotope, the longer half-life of fluorine-18 allows more time for radiosynthesis, longer scanning times, as well as the opportunity to transport the radiotracer to neighboring PET facilities. Furthermore, the previous work based their analysis on a racemic radiotracer, and the (*R*)-enantiomer of HX-CH 44 is not active.[15] Our goal is to assess HX-CH 44 derivatives as potential $^{18}F$-labeled PET imaging probes.

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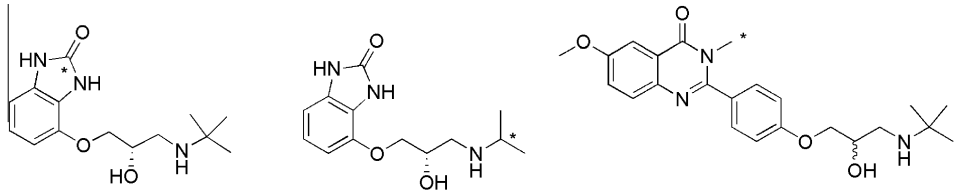



**Figure 1.** Chemical structures of [$^{11}$C]-CGP 12177, [$^{11}$C]-CGP 12388, and [$^{11}$C]-HX-CH 44. Asterisk denotes site of $^{11}$C.

The present study reports: (1) the synthesis of a racemic *N*-isopropyl derivative, as well as racemic and the 2*S*-enantiomer of *N*-fluoroisopropyl derivatives of HX-CH 44; (2) an efficient route to the core structure, 2-(4-hydroxyphenyl)-6-methoxy-3-methylquinazolin-4(*3H*)-one; (3) in vitro binding analyses of these compounds to assess their potency towards $\beta_1$-adrenergic receptors and selectivity over $\beta_2$-adrenergic and $5HT_{1A}$ receptors; and (4) the X-ray crystal structure of the fluorinated (*2S*)-derivative.

New derivatives of HX-CH 44 were prepared, incorporating 1-isopropylamino group ((*R*, *S*)-**6**) and 1-fluoroisopropylamino groups ((2*R*, 2*S*)-**7** and (2*S*)-**7**), into the respective 3-phenoxy-2-propanol moiety (Scheme 1). The HX-CH 44 core was prepared from 4-(benzyloxy)benzoic acid, that was converted to an acid chloride with thionyl chloride, and reacted directly with 2-amino-5-methoxybenzoic acid, to prepare the corresponding benzooxazin-4-one (**1**; 62%), from which the *N*-methylbenzamide (**2**) was prepared by reaction with methylamine in 97% yield. Ring-closure of **2**, by condensation of amides, was achieved under basic conditions by heating with KOH to afford the 4(*3H*)-quinazolinone (**3**) in 87% yield. Hydrogenolysis of the benzyl ether to liberate the free phenol, yielding the HX-CH 44 core (**4**), was nearly quantitative (97%). The highly efficient and concise synthesis of **4** is generally applicable for the preparation of arrays of structurally related quinazolinones. The core compound was used to generate both the racemic and enantiopure epoxides, (*R,S*)-**5** (82%) and (*S*)-**5** (92%), by reactions with epichlorohydrin or (*S*)-(+)-glycidyl nosylate, respectively. Compound **6** was efficiently prepared by a

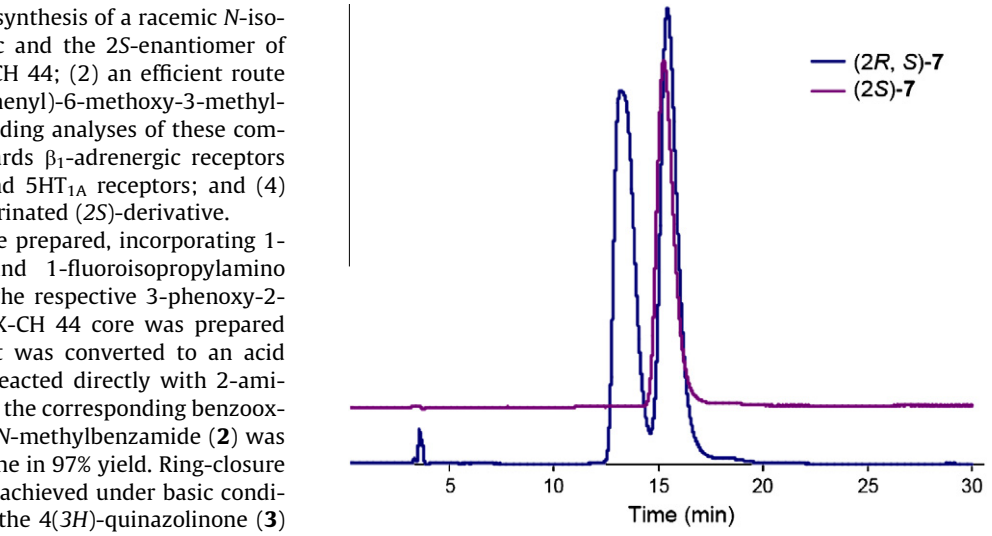
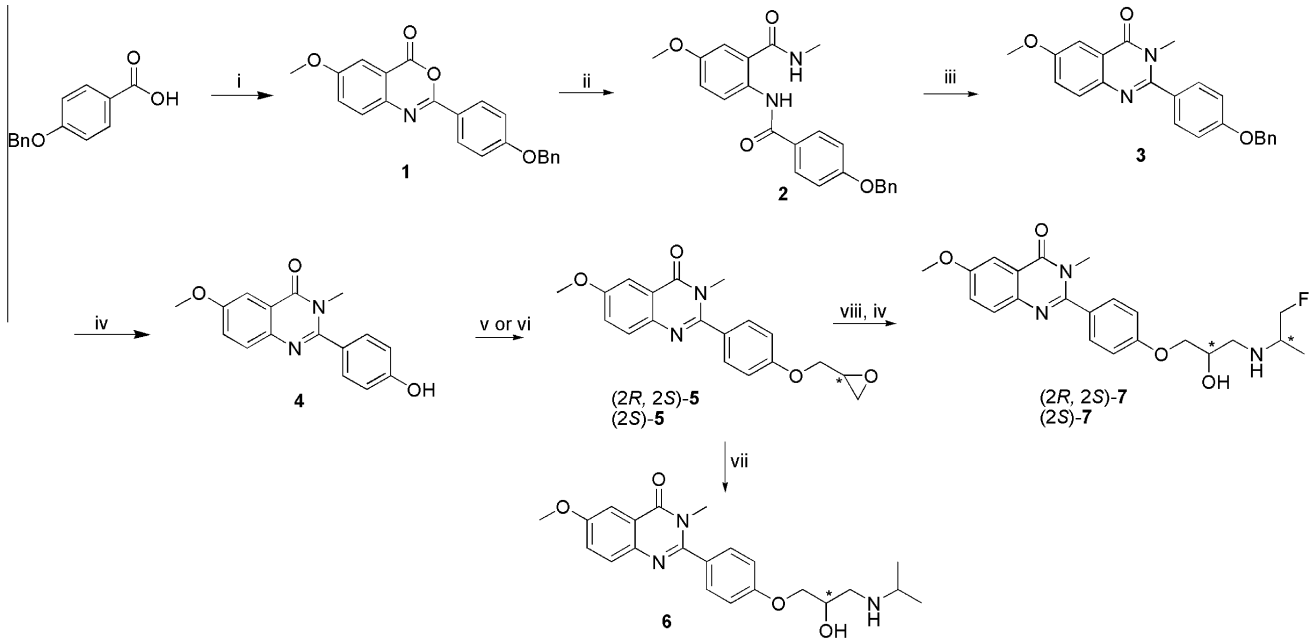



**Figure 2.** Chiral HPLC of **7** (Chiralcel® OD (250 × 4.6 mm) column, 70/30/0.1 hexanes/ethyl acetate/diethylamine, 1.0 mL/min, $\lambda$ = 254 nm).




**Scheme 1.** Synthesis **4**, **6**, (2*R*, 2*S*)-**7** and (2*S*)-**7**. Conditions: (i) (1) $SOCl_2$ (2) 2-amino-5-methoxybenzoic acid, $NEt_3$, 62% (ii) methylamine (33 wt % in EtOH), 97% (iii) KOH, 97% (iv) Pd/C, $H_2$, 97–100% (g); (v) epichlorohydrin, KOH, DMSO, 82% or (vi) (*S*)-(+)-glycidyl nosylate, $K_2CO_3$, CsF, 92% (vii) isopropylamine, 69% (viii) *N*-benzyl-1-fluoropropan-2-amine, 38% from (*R,S*)-**5**; (*2R, 2S*)-**7**, 45% from (2*S*)-**5**; (2S)-**7**, >99% de with respect to carbon-2. Asterisk denotes chiral center.

**Table 1**
In vitro binding affinities[a] ($K_i$ values in μM) for (2*R*, 2*S*)-**6**, (2*R*, 2*S*)-**7** and (2*S*)-**7** towards $\beta_1$, $\beta_2$ and 5-$HT_{1A}$ receptors

| | $\beta_1$ | $\beta_2$ | 5-$HT_{1A}$ |
|---|---|---|---|
| (2*R*, 2*S*)-**6** | 2.4 ± 0.4 | 7.7 ± 2.2 | 5.5 ± 1.0 |
| (2*R*, 2*S*)-**7** | 2.3 ± 0.4 | >10 | >10 |
| (2*S*)-**7** | 3.0 ± 0.6 | >10 | >10 |

[a] $K_i$ determinations was generously conducted by the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract # NO1MH32004 (NIMH PDSP). The NIMH PDSP is Directed by Bryan L. Roth MD, PhD at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscol at NIMH, Bethesda MD, USA. For experimental details please refer to the PDSP web site http://pdsp.med.unc.edu/ and click on 'Binding Assay' on the menu bar.

ring-opening reaction of the racemic epoxide (*R, S*)-**5**, with isopropylamine in 69% yield. As per our recent successes in synthesizing fluorinated β-blockers, fluoro-(2*S*)-toliprolol[9] and fluoro-(2*S*)-exaprolol,[8] the fluorinated derivatives in the present work were similarly prepared by effecting ring-opening of the epoxides, (*R, S*)-**5** and (*S*)-**5** by *N*-benzyl protected fluoroisopropylamine.[18] This convergent approach is straightforward and following hydrogenolysis yielded the fluorinated standard compounds (2*S*)-**7** and (2*R*, 2*S*)-**7** in moderate yields (38% and 45%, respectively). The diastereomeric excess (de) of (2*S*)-**7** at carbon-2 was >99%, as determined using chiral HPLC
(Fig. 2). Traditionally, the 2-hydroxyl group of the 1-isopropylamino-3-phenoxy-2-propanol moiety is on a stereogenic carbon for which the 2*S*-enantiomer has higher affinity for the receptor than the 2*R*-enantiomer.[19,20] Therefore, both racemic and (2*S*)-*N*-fluoroisopropyl (±)HX-CH 44 derivatives, ((2*R*, 2*S*)-**7** and (2*S*)-**7**) were synthesized and evaluated biologically. No attempt was made to assign the stereochemistry at carbon-5 because it has been shown that β-blockers fluorinated on the *N*-isopropyl chain do not show diastereomeric differences in metabolism or binding in vivo.[21]

Compounds (*R, S*)-**6**, (2*R*, 2*S*)-**7** and (2*S*)-**7** were assayed for their in vitro binding affinities towards $\beta_1$-adrenergic, $\beta_2$-adrenergic receptors (Table 1). A study by Roth and coworkers[22] speculated concerns of 5-$HT_{1A}$ affinity with a fluorinated β-blocker used in PET ([$^{18}$F]-fluoro-(2*S*)-Carazolol), therefore all of these compounds were also assayed for 5-$HT_{1A}$ affinity. Whereas (*R, S*)-**6** appeared unselective towards all three targets in vitro, both the fluorinated analogs, (2*R*, 2*S*)-**7** and (2*S*)-**7**, appeared to have high selectivity towards $\beta_1$-ARs. Fluorination of the isopropylamine moiety has been shown to increase the lipophilicity of β-blockers,[23] likely due to decreased basicity of the nitrogen,[24,25] and may be responsible for this enhanced biological behavior. Unfortunately, all three compounds have $K_i$ values between 2 and 3 μM towards $\beta_1$-ARs and is likely too high to be useful as leads for PET, particularly for brain imaging where the β-AR density (Bmax) in the frontal cortex of the human brain is low (18–147 fmol/mg of protein).[26,27] Previous work,[6,7] including ours,[8] has demonstrated that radiotracers with much higher affinities (picomolar) are likely required to successfully image β-ARs in humans.

Computer-aided design using the high resolution crystal structures of β-ARs has been an area of active pursuit in drug discovery.[28] We report herein, the X-ray crystal structure of (2*S*)-**7** (Fig. 3). Our laboratory has previously determined the X-ray structures of *N*-isopropylbenzamide[29] and its *N*-fluoroisopropyl analog.[30] The present structure complements our previous work by reporting the first crystallographic characterization of the 1-fluoroisopropylamino-3-phenoxy-2-propanol moiety that is common to several $^{18}$F-labeled β-blockers and may provide insights into development of potent and selective β-blockers. Our future work will continue to synthesize candidate radiopharmaceuticals to image β-ARs based on compounds with significantly higher affinities towards these targets, and we anticipate that new candidates for radiolabeling will be unveiled for this important target in the near future.

In conclusion, *N*-isopropyl and *N*-fluoroisopropyl derivatives of HX-CH 44 were efficiently synthesized and the synthetic strategy is generally applicable to preparing arrays of 4(3*H*)-quinazolinones. In vitro binding assays revealed that although the fluorinated analogs were selective towards $\beta_1$-adrenergic receptors they were not potent enough ($K_i$ = 2 – 3 μM) to warrant radiolabeling with fluorine-18. The X-ray crystal structure of the (2*S*)-fluorinated analog may offer new opportunities to develop higher affinity β-blockers via computer-aided drug design.

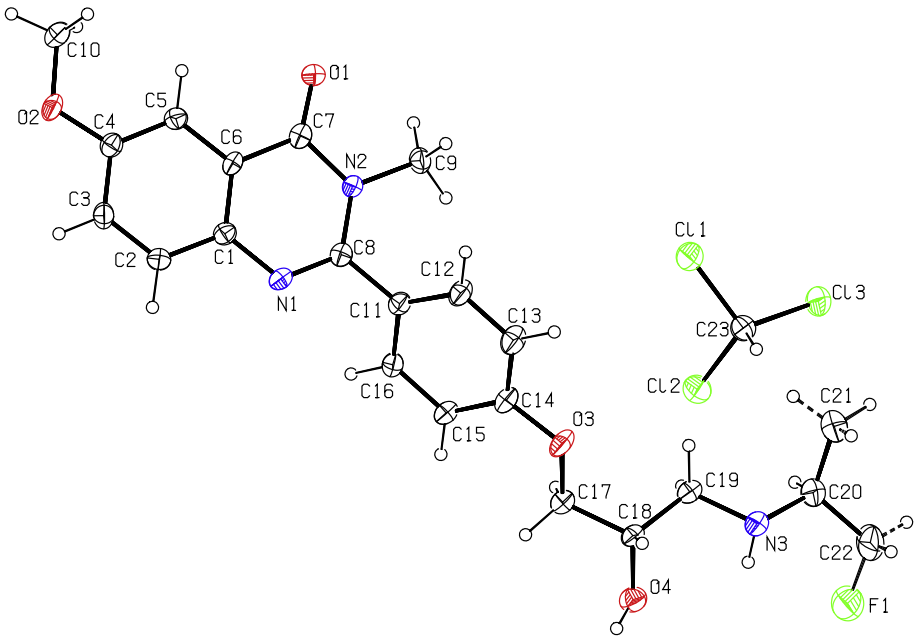



**Figure 3.** X-ray structure of (2*S*)-**7**·$CHCl_3$. The structure is disordered about the $CH_3$ and $CH_2F$ groups with occupancies of 30% and 70%, respectively.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.06.106.

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