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Synthesis and biological characterization of 3-(imidazol-1-ylmethyl)piperidine sulfonamides as aromatase inhibitors

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ABST RACT

The most frequently used treatment for hormone receptor positive breast cancer in post-menopausal women are aromatase inhibitors. In order to develop new aromatase inhibitors, we designed and synthesized new imidazolylmethylpiperidine sulfonamides using the structure of the previously identified aromatase inhibitor SYN 20028567 as starting lead. By this approach, three new aromatase inhibitors with IG_{50} values that are similar to that of letrozole and SYN 20028567 were identified.

The standard treatment of estrogen receptor positive (ER+) breast cancer is endocrine therapy.[1] Selective estrogen receptor modulators (SERMs),[2] such as tamoxifen, are one of the backbones of endocrine therapy in breast cancer. They exert their action by blocking the binding of estrogen to ERs. Indeed, in post-menopausal women, the main category affected by this disease, estrogens are produced in breast or, generally, in nonovarian tissues, mainly in adipose tissue. However, some undesirable adverse effects of SERMs, due to the their residual estrogenic activities, such as the increased risk of endometrial cancer or of thromboembolism, made necessary the development of other therapies. Since cytochrome P450 aromatase (CYP19) plays a key role in the biosynthesis of estrogens, being responsible for the aromatization of androgens such as androstenedione and testosterone, aromatase inhibitors (AIs) were eventually developed and these rapidly proved to be an important alternative to SERMs.[3] AIs do not show estrogenic effects, they have a different spectrum of adverse effects compared to SERMs, primarily consisting of bone pains and ostheoporosis and, most importantly, they have a demonstrated efficacy in the treatment of early- and advanced-stage breast cancer in post-menopausal women. Essentially, the AIs that are currently in clinical use consist of the non-steroidal AIs, anastrozole and letrozole, and of the steroidal AI, exemestane (Fig. 1).[4] Despite the efficacy of these two families of AIs, the medicinal chemists are continuously making efforts to develop new and effective AIs with more clinical efficacy and minimal side effects.[5] In a previous work, we took advantage of the recently published X-ray structure of aromatase to develop a high throughput docking (HTD) protocol for the identification of new AIs.[6] Its application to the virtual screening of a set of commercially available organic molecules led to the identification of novel non-steroidal AIs with scaffolds remarkably different compared to the one of anastrozole and letrozole.

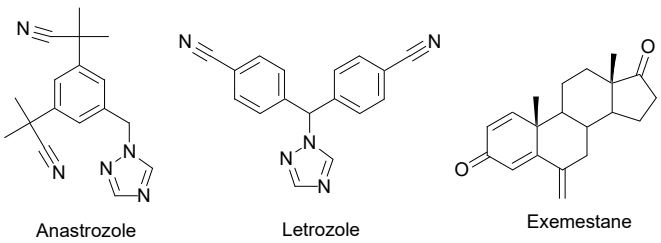


Figure 1. Aromatase inhibitors.

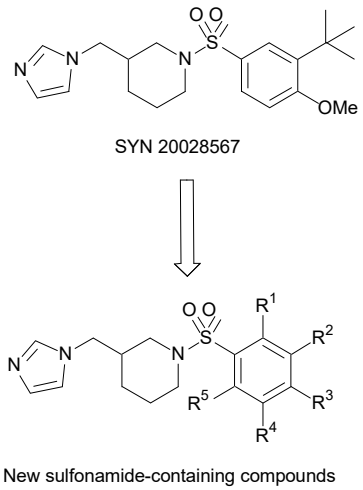


Figure 2. SYN 20028567 and new sulfonamide-containing compounds.

In particular, an imidazolylmethylpiperidine sulfonamide derivative (SYN 20028567, Fig. 2) proved to be the most active compound in enzymatic assays. Many other classes of imidazolylmethyl-substituted inhibitors of steroidogenic CYP enzymes have been described, displaying potent inhibitory activity.[7]

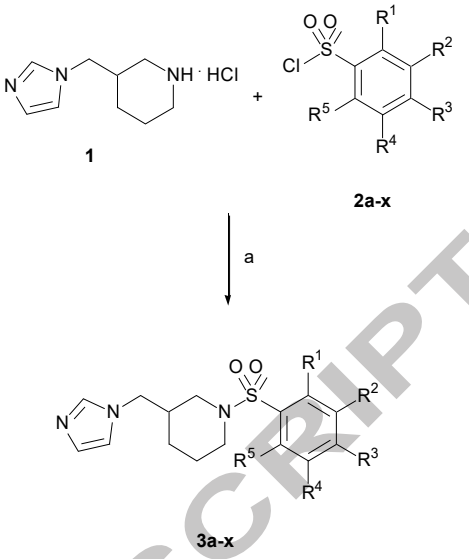
Herein, we describe the optimization of this promising non-steroidal lead compound. Three features of the whole frame of compound SYN 20028567 were maintained: the imidazole, which is necessary for the coordination of the heme iron,[8] the piperidine, which was preferred to morpholine since derivatives of the latter proved less active in the previous study and, finally, the sulfonyl portion, which was preferred to a carbonyl one, because it undergoes an additional hydrogen bond interaction with the protein Modifications of the original scaffold SYN 20028567 were primarily performed by varying the substituents on the aromatic ring, selected among commercially available compounds (Fig. 2).

For this reason, a series of commercially available sulfonylchlorides (**2a-x**) was reacted with imidazolylpiperidine cloridrate **1** in dry dichloromethane, at 0 °C for 2 h and then at room temperature (rt) for 4 days. The aromatic sulfonamides **3a-x** were purified by column chromatography on silica gel (Scheme 1).

To further differentiate the obtained scaffold, sulfonamides **3l** and **3q** were converted into sulfonamides **3y** and **3z** by a nucleophilic substitution of the fluorine atom (Scheme 2). Cyclopentanol and **3l** or **3q** were added to a suspension of NaH in dry THF at rt, under nitrogen atmosphere. After reflux for 24 h, the crude products **3y** and **3z** were purified on silica gel.

Overall, 26 sulfonamides were synthesized varying the substituents on the five free positions of the aromatic ring. Similar to the lead SYN 20028567, all the compounds were obtained and tested in racemic form.

The aromatase inhibitory activity of the new compounds was determined according to a semiautomated high-throughput screening method, which employs recombinant human aromatase and a fluorometric substrate, 7-methoxy-4-trifluoromethyl coumarin (MFC), by measuring the reduction in fluorescence associated with the reaction of the MFC.[9] Letrozole and SYN 20028567 were used as controls and indeed showed inhibitory

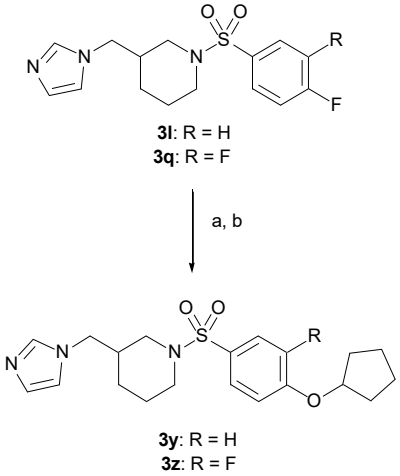


Scheme 1. Reagent and conditions: a) NEt_3, dry CH_2Cl_2, 0 °C to rt, 4 days.

activities, in line with those described in the literature (4 and 9 nM, respectively).[10] The degree of inhibition obtained with the different compounds was normalized to that obtained without inhibitor addition. Results are summarized in Table 1.

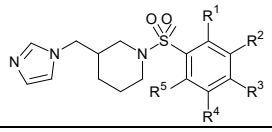
The tested compounds exhibited moderate to excellent aromatase inhibition, with IC_{50} values in the nanomolar range. The most potent AIs were **3d**, **3h**, and **3o**, with IC_{50} values of 9, 7, and 6 nM, respectively.[11] These IC_{50}s are very close to those of letrozole and the lead compound SYN 20028567. The less active compounds were found to be **3i**, **3j**, **3m**, and **3s**. The other molecules of our series showed intermediate values of enzyme inhibition.

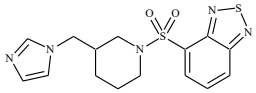
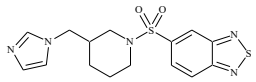
These results showed that R^2 substituents, such as halogens or trifluoromethyl, played a key role in determining the inhibitory activity of our compounds, leading to IC_{50} values that ranged between 6 and 46 nM (**3c**, **3e**, **3n**, **3o**, **3u**, and **3y**). Even when R^2 was a more hindered substituent (**3d**), and also including the position R^3, a high potency was observed, with IC_{50} values ranging between 9 and 54 nM (**3f**, **3g**, **3h**). The structural motifs characterizing the investigated molecules, such as the sulfonamide group, the piperidine and the imidazole, are important in conferring the compounds' inhibitory activity.



Scheme 2. Reagent and conditions: a) cyclopentanol, NaH, dry THF, rt, 0.5 h; b) THF, reflux, 24 h.

Table 1. Inhibition of human aromatase for the new compounds **3a-z**. [a,b]



Compd	R^1	R^2	R^3	R^4	R^5	IC_{50} (nM)
3a	$COOCH_3$	H	H	H	H	53
3b	H	CH_3	F	H	H	24
3c	H	F	OCH_3	H	H	14
3d	H		H	H	H	9
3e	H	Cl	F	H	H	14
3f	H	OCH_2CH_2O		H	H	13
3g						54
3h						7
3i	H	COOH	OH	H	H	339
3j	H	H	OCH_3	H	OCH_3	131
3k	H	H	CN	H	Cl	79
3l	H	F	F	H	H	43
3m	H	H	CN	H	H	36
3n	F	F	F	H	H	46
3o	H	Br	OCH_3	H	H	6
3p	H	H	OCH_3	OCH_3	H	42
3q	H	H	F	H	H	51
3r	H	H	F	H	CH_3	48
3s	OCH_3	H	NO_2	H	H	129
3t	H	H	SO_2CH_3	H	H	48
3u	H	CF_3	NO_2	H	H	16
3v	CH_3	CH_3	OCH_3	CH_3	H	45
3w	H	CH_3	OCH_3	CH_3	H	21
3x	CH_3	CH_3	OCH_3	H	H	59
3y	H	F		H	H	22
3z	H	H		H	H	14
letrozole						4
SYN 20028567						9

[a]The reaction was conducted in anoxic conditions. [b]Compounds were tested in DMSO, at maximum concentration of 25 μM. Assays were performed in duplicate. Blank values were subtracted from the sample wells to obtain the net fluorescence signal. The values were normalized to those obtained without inhibitor addition.

In addition, moreover, specific substitutions in R^2 and R^3 seem to favorably affect the *in vitro* profile.

In summary, in this letter we report on 26 non-steroidal sulfonamide-containing compounds that were synthesized starting from a previously identified AI, the imidazolylmethylpiperidine sulfonamide SYN 20028567. These compounds can be easily prepared in a one-step chemical synthesis from readily available imidazolylpiperidine cloridrate and commercial sulfonylchlorides with high yields. The most potent molecules in terms of aromatase inhibition were **3d**, **3h**, and **3o**, which we found to have IC_{50} values in the low nanomolar range and essentially similar to those of letrozole and of the lead compound, SYN 20028567. Further biological studies of these newly identified AIs are currently underway, mainly focused on selectivity towards other steroidogenic CYP enzymes, and additional derivatives based on the same pharmacophore are in the process of being synthesized and tested.

Acknowledgments

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References and notes

1. a) Cigler, T.; Goss, P. E. *Cancer J.* **2007**, *13*, 148; b) Yamashita, H. *Int. J. Clin. Oncol.* **2008**, *13*, 380; c) Sainsbury, R. *Cancer Treat. Rev*. **2013**, *39*, 507.
2. a) Komm, B. S.; Mirkin, S. *J. Steroid Biochem. Mol*. **2014**, *143*, 207; b) Musa, M. A.; Khan, M. O. F.; Cooperwood, J. S. *Curr. Med. Chem*. **2007**, *14*, 1249.
3. a) Carpenter, R.; Miller, W. R. *Br. J. Cancer* **2005**, *93* (SUPPL. 1), S1; b) Brueggemeier, R. W.; Hackett, J. C.; Diaz-Cruz, E. S. *Endocr. Rev*. **2005**, *26*, 331.
4. O'Reilly, J. M.; Brueggemeier, R. W. *Curr. Med. Chem*. **1996**, *3*, 11-22; b) Bruno, R. D.; Njar, V. C. O. *Bioorg. Med. Chem*. **2007**, *15*, 5047.
5. a) Ahmad, I.; Shagufta. *Eur. J. Med. Chem*. **2015**, *102*, 375; b) Wang, R.; Shi, H.-F.; Zhao, J.-F.; He, Y.-P.; Zhang, H.-B.; Liu, J.-P. *Bioorg. Med. Chem. Lett*. **2013**, *23*, 1760; c) McNulty, J.; Keskar, K.; Crankshaw, D. J.; Holloway, A. C. *Bioorg. Med. Chem. Lett*. **2014**, *24*, 4586.
6. Caporuscio, F.; Rastelli, G.; Imbriano, C.; Del Rio, A. *J. Med. Chem*. **2011**, *54*, 4006.
7. a) Lina Yin, L.; Lucas, S.; Maurer, F.; Kazmaier, U.; Qingzhong Hu, Q.; Hartmann, R. W. *J. Med. Chem*. **2012**, *55*, 6629; b) Abadi, A. H.; Abou-Seri, S. M.; Hu, Q.; Negri, M.; Hartmann, R. W. *Med. Chem. Commun.* **2012**, *3*, 663; c) Ranju Bansal, R.; Narang, G Zimmer, C.; Hartmann, R. W. *Med. Chem. Res*. **2011**, *20*, 661.
8. Rahman, M. N.; Vlahakis, J. Z.; Roman, G.; Vukomanovic, D.; Szarek, W. A.; Nakatsu, K.; Jia, Z. *J. Inorg. Biochem*. **2010**, *104*, 324.
9. Stresser, D. M.; Turner, S. D.; McNamara, J.; Stocker, P.; Miller, V. P.; Crespi, C. L.; Patten, C. J. *Anal. Biochem*. **2000**, *284*, 427.
10. Schuster, D.; Laggner, C.; Steindl, T. M.; Palusczak, A.; Hartmann, R. W.; Langer, T. *J. Chem. Inf. Model*. **2006**, *46*, 1301.
11. Data for **3d**, **3h**, and **3o**. **3d**: crystalline white solid, 61% yield, mp 91-92 °C. IR (KBr) 3096, 3937, 1583, 1533, 1451 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.08-1.19 (m, 1H), 1.55-1.66 (m, 2H), 1.73-1.81 (m, 1H), 2.02-2.12 (m, 1H), 2.44-2.50 (m, 1H), 2.70-2.77 (m, 1H), 3.21-3.29 (m, 2H), 3.77-4.02 (dq, 2H, J = 7.2 Hz), 6.90 (broad s, 1H), 6.95 (d, 2H, J =.7 Hz), 7.06 (broad s, 1H), 7.43 (t, 1H, J = 8.1 Hz), 7.44 (broad s, 1H), 7.67 (d, 2H, J = 8.7 Hz), 7.78 (dd, 1H, J = 8.1 Hz, J = 1.5 Hz), 7.95 (dd, 1H, J = 8.1 Hz, J = 1.5 Hz); ^{13}C NMR ($CDCl_3$) δ 23.3, 27.2, 37.0, 46.9, 48.9, 49.3, 100.3, 116.0, 119.4, 124.5, 127.0, 130.0, 130.8, 131.1, 135.8, 137.7, 143.6, 144.8, 160.2. Anal Calcd for $C_{21}H_{21}ClN_4O_5S$: C, 52,858; H, 4,436; N, 11,794. Found C, 52,951; H, 4,427; N, 11,775. **3h**: hygroscopic white solid, 72% yield, mp 164-165 °C. IR (KBr) 3108, 2935, 1669, 1511, 1450 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.06-1.18 (m, 1H), 1.57-1.69 (m, 2H), 1.75-1.84 (m, 1H), 2.06-2.16 (m, 1H), 2.54-2.61 (m, 1H), 2.80-2.88 (m, 1H), 3.33-3.40 (m, 2H), 3.78-4.04 (dq, 2H, J = 6.9 Hz), 6.90, 7.06 and 7.43 (broad s, 3H), 7.84 (dd, 1H, J = 9.0 Hz, J = 2.1 Hz), 8.14 (d, 1H, J = 9.0 Hz), 8.48 (d, 1H, J = 0.9 Hz); ^{13}C NMR ($CDCl_3$) δ 23.4, 27.2, 37.0, 47.0, 49.0, 49.2, 119.4, 122.7, 123.0, 126.4, 130.1, 137.7, 137.8, 153.8, 155.8. Anal Calcd for $C_5H_{17}N_5O_2S_2$: C, 49,527; H, 4,711; N, 19,339. Found C, 49,411; H, 4,708; N, 19,321. **3o**: white solid, 72% yield, mp 105-106 °C. IR (KBr) 3108, 2938, 2849, 1583, 1484, cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.08-1.19 (m, 1H), 1.56-1.65 (m, 2H), 1.71-1.82 (m, 1H), 2.05-2.15 (m, 1H), 2.45-2.51 (m, 1H), 2.71-2.79 (m, 1H), 3.19-3.27 (m, 2H), 3.79-4.05 (dp, 2H, J = 7.2 Hz), 3.97 (s, 3H), 6.91 (broad s, 1H), 6.98 (d, 1H, J = 8.7), 7.08 (broad s, 1H), 7.44 (broad s, 1H), 7.65 (dd, 1H, J = 8.7, J = 2.4), 7.90 (d, 1H, J = 2.4); ^{13}C NMR ($CDCl_3$) δ 23.2, 27.2, 36.9, 46.9, 48.9, 49.3, 56.9, 111.7, 112.6, 119.5, 128.9, 129.0, 130.1, 132.7, 137.7, 159.6. Anal Calcd for $C_{16}H_{20}BrN_3O_3S$: C, 46,363; H, 4,864; N, 10,183. Found C, 46,283; H, 4,851; N, 10,149.

Supplementary Material

Supplementary data (chemistry and biology) associated with this article can be found, in the online version, at ……

Graphical Abstract

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