Accepted Manuscript

Design, synthesis, and evaluation of (2S, 4R)-Ketoconazole sulfonamide analogs as potential treatments for metabolic syndrome

Benjamin E Blass, Pravin Iyer, Magid Abou-Gharbia, Wayne W Childers, John C Gordon, Mercy Ramanjulu, George Morton, Premkumar Arumugam, Joshodeep Boruwa, John Ellingboe, Sayan Mitra, Rajashekar Reddy Nimmareddy, Shalini Paliwal, Jamallamudi Rajasekhar, Savithiri Shivakumar, Pratima Srivastava, Raghuram S. Tangirala, Konda Venkataramanaiah, Mahesh Yanamandra

PII: S0960-894X(16)31045-9
DOI: http://dx.doi.org/10.1016/j.bmcl.2016.10.016
Reference: BMCL 24319

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 25 June 2016
Revised Date: 3 September 2016
Accepted Date: 7 October 2016

Please cite this article as: Blass, B.E., Iyer, P., Abou-Gharbia, M., Childers, W.W., Gordon, J.C., Ramanjulu, M., Morton, G., Arumugam, P., Boruwa, J., Ellingboe, J., Mitra, S., Nimmareddy, R.R., Paliwal, S., Rajasekhar, J., Shivakumar, S., Srivastava, P., Tangirala, R.S., Venkataramanaiah, K., Yanamandra, M., Design, synthesis, and evaluation of (2S, 4R)-Ketoconazole sulfonamide analogs as potential treatments for metabolic syndrome, *Bioorganic & Medicinal Chemistry Letters* (2016), doi: http://dx.doi.org/10.1016/j.bmcl.2016.10.016

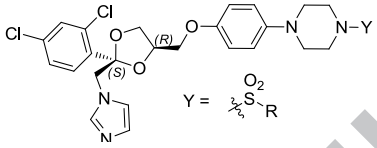
Graphical Abstract

To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

Design, synthesis, and evaluation of (2S, 4R)-Ketoconazole sulfonamide analogs as potential treatments for metabolic syndrome

Leave this area blank for abstract info.

Benjamin E Blass, Pravin Iyer, Magid Abou-Gharbia, Wayne W Childers, John C Gordon, Mercy Ramanjulu, George Morton, Premkumar Arumugam, Joshodeep Boruwa, John Ellingboe, Sayan Mitra, Rajashekar Reddy Nimmareddy, Shalini Paliwal, Jamallamudi Rajasekhar, Savithiri Shivakumar, Pratima Srivastava, Raghuram S. Tangirala, Konda Venkataramanaiah, and Mahesh Yanamandra.



Bioorganic & Medicinal Chemistry Letters
journal homepage: www.elsevier.com

Design, synthesis, and evaluation of (2S, 4R)-Ketoconazole sulfonamide analogs as potential treatments for metabolic syndrome

Benjamin E Blass[a] , * Pravin Iyer [b], Magid Abou-Gharbia[a], Wayne W Childers[a], John C Gordon[a], Mercy Ramanjulu[a], George Morton[a], Premkumar Arumugam[b], Joshodeep Boruwa[b], John Ellingboe[b], Sayan Mitra[b], Rajashekar Reddy Nimmareddy[b], Shalini Paliwal[b], Jamallamudi Rajasekhar[b], Savithiri Shivakumar[b], Pratima Srivastava[b], Raghuram S. Tangirala[b], Konda Venkataramanaiah[b], and Mahesh Yanamandra[b]

[a]*Moulder Center for Drug Discovery Research, Temple University School of Pharmacy, 3307 N Broad Street, Philadelphia, PA 19140 USA.*
[b]*GVK Biosciences Private Limited, Plot 28A, IDA Nacharam, Hyderabad, India 500076.*

ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Keywords:
Metabolic syndrome
Ketoconazole
Cortisol
17α-hydroxylase-C17,20-lyase (Cyp17)

ABSTRACT

Metabolic syndrome, also referred to as "Syndrome X" or "Insulin Resistance Syndrome," remains a major, unmet medical need despite over 30 years of intense effort. Recent research suggests that there may be a causal link between this condition and abnormal glucocorticoid processing. Specifically, dysregulation of the hypothalamic-pituitary-adrenocortical (HPA) axis leads to increased systemic cortisol concentrations. Cushing' syndrome, a disorder that is also typified by a marked elevation in levels of cortisol, produces clinical symptomology that is similar to those observed in MetS, and they can be alleviated by decreasing circulating cortisol concentrations. As a result, it has been suggested that decreasing systemic cortisol concentration might have a positive impact on the progression of MetS. This could be accomplished through inhibition of enzymes in the cortisol synthetic pathway, 11β-hydroxylase (Cyp11B1), 17α-hydroxylase-C17,20-lyase (Cyp17), and 21-hydroxylase (Cyp21). We have identified a series of novel sulfonamide analogs of (2S, 4R)-Ketoconazole that are potent inhibitors of these enzymes. In addition, selected members of this class of compounds have pharmacokinetic properties consistent with orally delivered drugs, making them well suited to further investigation as potential therapies for MetS.

∗ Corresponding author. Tel.: +1-215-707-2218; fax: +1-215-707-5296; e-mail: Benjamin.Blass@Temple.edu

Over the course of the last three decades, a growing body of knowledge has been developed to describe Meta-bolic Syndrome (MetS), also referred to as "Syndrome X" or "Insulin Resistance Syndrome."[1] MetS is defined as a cluster of abnormalities that occur in concert, including high blood pressure (BP), hyperglycemia, reduced high density lipoprotein cholesterol (HDL-C) levels, elevated triglycerides (TG) and abdominal obesity. The most widely accepted definition of this condition is based on the National Cholesterol Education Program (NCEP) Adult Treatment Panel-III (ATP-III), which provides for the diagnosis of MetS in patients that meet at least three of the following parameters identified in Table 1. Current estimates indicate that nearly 25% of the world's adult population suffers from MetS, and the incidence is rising, largely as a result of increased obesity rates.[2]

Table 1: Metabolic Syndrome diagnostic parameters

Parameter	Men	Women
Waist size	>102 cm	>88 cm
HDL-C	<40 mg/dL	<50 mg/dL
TG	>150 mg/dL	>150 mg/dL
BP	>130/85	>130/85
Fasting Glucose	>110 mg/dL	>110 mg/dL

While there are many factors that contribute to the development of MetS, there is strong support for the theory that abnormal glucocorticoid processing plays an important role in the progression of this condition. The glucocorticoids were originally named for their contribution to glucose metabolism, but it has since been shown that they play a much wider role in resting and stress related homeostasis. Cortisol in particular has been implicated in MetS by virtue of the high degree of similarity between the clinical manifestation of MetS and Cushing's syndrome. Much like MetS, the clinical manifestations of Cushing's syndrome include central obesity, hypertension, dyslipidemia, and insulin resistance that may manifest as impaired glucose tolerance or type 2 Diabetes.[3] Cushing's syndrome is also typified by a marked elevation in levels of cortisol. Surgical intervention at the pituitary or adrenal level leads to a marked decrease in centralization of fat deposits observed in patients with Cushing's syndrome. This result can be attributed to decreased levels of circulating cortisol as a result of the surgical intervention. In related findings, it has been demonstrated that exogenous treatment with glucocorticoids leads to the development of abdominal obesity in both animals and humans, further suggesting that glucocorticoids play an important role in the development of this phenotype. Also, animal models of obesity are known to demonstrate elevated levels of corticosterone (the equivalent of cortisol in humans).[4]

Taken together, these findings strongly suggest that modulation of glucocorticoid processing may be a viable approach to the treatment of MetS and related disorders. One possible method of correcting the dysregulation of the hypothalamic-pituitary-adrenocortical (HPA) axis associated with MetS is decreasing circulating cortisol level. This could be accomplished by inhibiting one or more of the enzymes in the cortisol synthetic pathway. Viable targets include 11β-hydroxylase (Cyp11B1), 17α-hydroxylase-C17,20-lyase (Cyp17),[5] and 21-hydroxylase (Cyp21).[6] All three are members of the cytochrome P450 superfamily of enzymes. Cyp11B1 catalyzes the final step of cortisol synthesis, hydroxylation of the C-11 position of deoxycortisol. Cyp17 has multiple functions in corticosteroid synthesis. The C-17 and C-20 positions of the steroid framework can be modified by this enzyme. Pregnenolone and progesterone are hydroxylated by Cyp17 at C-17 (hydroxylase activity), while the C-20/C-17 bond is cleaved by the same enzyme in 17α-hydroxyprogesterone and 17-hydroxypregnenolone (lyase activity). Finally, Cyp21 catalyzes the hydroxylation of C-21 in steroids such as progesterone and 17α-hydroxyprogesterone. Although there has been some progress in the identifying selective Cyp11B1 inhibitors,[7] none has reached the market. In addition, accumulation of cortisol precursors represents an added risk to Cyp11B1 inhibition. This risk could be avoided by targeting Cyp17 and/or Cyp21, each of which is important to cortisol synthesis.[8]

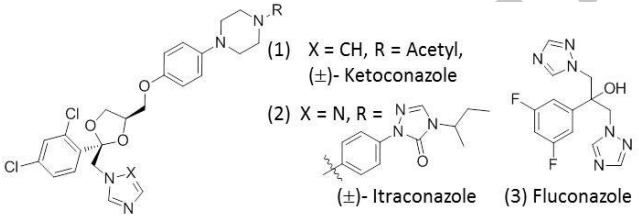


Figure 1: Marketed azole antifungal agents (1) (±)-Ketoconazole, (2) Itraconazol, (3) Fluconazole

Efforts to develop novel therapies for the treatment of MetS recently led to the identification of (±)-Ketoconazole (**1**) as an agent capable of lowering plasma cortisol concentrations. (±)-Ketoconazole was originally identified in 1979 by scientists at Janssen Pharmaceuticals as a potent antifungal agent.[9] It was the first orally active azole antifungal agent. Although itraconazole[10] (**2**) and fluconazole[11] (**3**) have proven to be safer and more effective for systemic fungal infections, (±)-Ketoconazole remains a major therapeutic tool for the treatment of fungal infections of the skin such as athlete's foot, ringworm, and candidiasis. The antifungal activity of (±)-Ketoconazole is driven by its ability to inhibit the synthesis of ergosterol, an important component of fungal cell membranes, by inhibiting Cyp450-α-demethylase.[12] (±)-Ketoconazole is also known as a potent inhibitor of Cyp3A4. As a result oral dosage forms are associated with a warning for potential drug-drug interaction with a wide range of important medications such as Imatinib, Simvastatin, Alprazolam, and Indinavir.[13]

Table 2: Ketoconazole Cyp17 and Cyp3A4 data

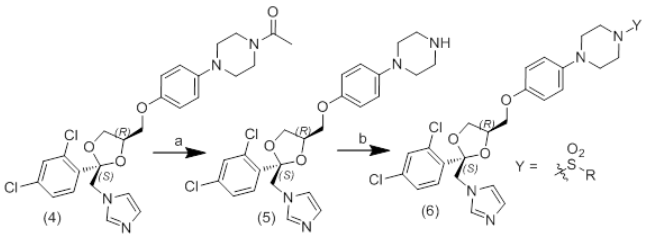
Compound	Cyp17*	Cyp3A4*	Ratio
	IC_{50} (nM)		
(±)-Ketoconazole	93	133	1.4
(2S, 4R)-Ketoconazole	48	146	3.0
(2R, 4S)-Ketoconazole	1800	136	0.076

* n = 3

Recently, it has been demonstrated that (±)-Ketoconazole is a potent inhibitor of Cyp17,[14] which, as noted above, is a key player in cortisol synthesis. In an effort to gain a better understanding of the relationship between cortisol synthesis and MetS, Per Marin et. al. examined the impact of 400 mg daily oral doses of (±)-Ketoconzaole in group of 30 female MetS patients. After 3 months, statistically significant improvements in multiple risk factors associated with MetS (insulin resistance, total cholesterol, fasting glucose levels, HbA1C levels, and blood pressure) were observed. These findings strongly support the application of cortisol synthesis inhibitors for the treatment of MetS, as well as the importance of Cyp17 as a therapeutic target for this condition.[15] The potential utility of (±)-Ketoconazole for the treatment of MetS is, however, limited by its potent inhibition of Cyp3A4. The MetS patient population is likely to be using multiple medications, raising the likelihood of adverse drug/drug interactions if (±)-Ketoconazole were to be added to the mix.

Interestingly, the individual enantiomers of (±)-Ketoconazole are not equally potent inhibitors of Cyp17.[16] Although the (2s, 4R) isomer potently inhibits Cyp17 (IC_{50} = 48 nM), its mirror image, the (2R, 4S) isomer, is much less potent (IC_{50} = 1800 nM). The same is not true, however, of their activity at Cyp3A4, as the two enantiomers are nearly equipotent at this key metabolic enzyme (Table 2). The difference in Cyp17 activity provides the (2s, 4R) isomer with a slightly better Cyp17/Cyp3A4 ratio, which may translate into a lower risk of drug/drug interactions in a clinical setting. In fact, this compound is currently the subject of on-going phase clinical studies for the treatment of endogenous Cushing's syndrome.[17] A positive result would provide patients in need with a new therapeutic option. Reducing the inhibition of CYP3A4 and thereby the risk of drug/drug interactions could be a further improve of this class of drugs.

Scheme 1: Synthesis of (2S, 4R)-Ketoconazole analogs 6a-6l



(a) NaOH, MeOH/H_2O (1/1), reflux 18 h. (b) RSO_2Cl, NEt_3, CH_2Cl_2, 25 °C, 18 h.

In an effort to address the Cyp3A4 issue, we launched a program designed to identify novel Cyp17 inhibitors with increased Cyp3A4 selectivity. As a first step on this road, we chose to focus on direct analogs of (2S, 4R)-Ketoconazole. To our surprise, there have been very few literature reports of (±)-ketoconazole analogs since its initial disclosure, and none focused on their potential as Cyp3A4 inhibitors. There were, however, a limited number of disclosures of the preparation of racemic deacylated (±)-Ketoconazole from the parent racemate.[18] Thus, deacylation of the enantiomerically pure (2S, 4R)-Ketoconazole provided us with easy access to a functionalizable piperazine nitrogen within the (2S, 4R)-Ketoconazole framework (**5**). Functionalization with a suitable sulfonyl chloride in the presence of trimethylamine provided rapid access to numerous analogs suitable for *in vitro* screening (Scheme 1).

As indicated in Table 3, replacing the acetyl group of (2S, 4R)-Ketoconazole (4) with a sulfonamide provides compounds with substantial inhibitory potency at Cyp17 (IC_{50} 67 – 0.73 nM). Potency at Cyp3A4 was also maintained, but to a lesser extent (IC_{50} 99 – 450 nM). In addition, differences in the SAR patterns of Cyp17 and Cyp3A4 enabled the identification of compounds with a higher degree of selectivity as compared to the parent compound (4). Installation of a methyl sulfonamide (6a), for example, produced a 3 fold increase in Cyp17 potency, while Cyp3A4 activity remained nearly unchanged. These differences produce a net effect of increasing Cyp17 selectivity from 3 fold to >10 fold. Extending the sulfonamide by one additional carbon atom (6b) lead to an additional >20 fold increase in Cyp17 potency (0.73 nM) and once again Cyp3A4 activity remained nearly unchanged (162 nM), leading to a 222 fold selectivity factor. The more sterically encumbered isopropyl sulfonamide (6c) was less potent at Cyp17 (12 nM) than 6b and therefore less selective versus Cyp3A4 (16.5 fold). Reducing the steric footprint of the isopropyl sulfonamide by incorporating the corresponding cyclopropyl ring restores some of the Cyp17 activity (3.7 nM) and Cyp3A4 selectivity (50 fold). Further elongation of the sulfonamide, as seen in the methoxyethyl analog (6e), led to an increase of Cyp17 potency (19 nM). Cyp3A4 potency, however, was little changed, and as a result the corresponding improvement of Cyp17/3A4 selectivity was moderate (9.7 fold). Restricting the flexibility of the methyl ether of **6e** via closure of a six membered ring led to an increase in Cyp17/3A4 selectivity (20.5 fold), as Cyp17 potency increased (11 nM) while Cyp3A4 potency decreased (226 nM).

Table 3: Physicochemical and *in vitro* screening results of exemplary (2S, 4R)-Ketoconazole analogs.

Entry	R	MW	cLogP	TPSA	Cyp17*	Cyp11*	Cyp21*	Cyp19*	Cyp3A4*	Cyp3A4/Cyp17	GPLM**
					IC_{50} (nM)						$t_{1/2}$ (min)
4	NA	531	5.2	69	48	116	4300	1600	146	3.0	45
6a	Me	567	4.1	86	15	500	1600	970	158	10.5	25
6b	Et	581	4.5	86	0.73	63	271	1600	162	222	25
6c	i-Pr	595	4.9	86	12	40	51	1200	198	16.5	33
6d	cyc-Pr	593	4.6	86	3.7	58	28	1820	185	50	19
6e	CH2CH2OMe	611	3.9	95	19	110	166	10000	185	9.7	56
6f		637	4.5	95	11	187	123	1240	226	20.5	15
6g	CF3	621	5.4	86	67	32	49	790	336	5.0	22
6h	CHF2	603	4.8	86	8	29	26	3800	99	12.4	30
6i	CH2CN	592	3.6	110	9	70	78	2800	153	17.0	51
6j	CH2SO2Me	645	3.4	120	7	67	20	1000	151	21.6	25
6k		635	6.1	86	9	82	148	10000	87	9.7	21
6l		633	5.3	104	15	17	8	10000	145	9.7	18

* n = 3, **GPLM = guinea pig liver microsome

Incorporation of electron withdrawing substituents into the sulfonamide group also produced interesting results. Selectivity for Cyp17 versus Cyp3A4 was slightly improved (5 fold selectivity) by replacing the methyl of **6a** with a trifluormethyl group (**6g**) as a result of a moderate increase in Cyp17 potency (67 nM) and a moderate decrease in Cyp3A4 potency (336 nM). Greater improvement in selectivity was observed with the difluoromethyl (**6h**), cyanomethyl (**6i**), and methylsulfonylmethyl (**6j**) analogs. In each of these three examples, Cyp17 potency increased by a factor of 5, while Cyp3A4 potency remained comparable to that observed with (2S, 4R)-Ketoconazole (**4**). Similar effects are observed with the incorporation of a 2-thiophene (**6k**) and N-methyl-2-imidazole (**6l**).

The impact of the sulfonamide analogs on three other enzymes that are critical to the synthesis of glucocorticoid processing, Cyp11B1, Cyp21, and Cyp19, was also explored. As indicated in table 3, (2S, 4R)-Ketoconazole (**4**) is a nearly as potent an inhibitor of Cyp11B1 as Cyp17 (116 nM vs. 48 nM). The majority of the corresponding sulfonamide derivatives examined were also potent Cyp11B1 inhibitors. While the installation of a methyl sulfonamide (**6a**) produces a >4-fold decrease in Cyp11 potency (500 nM), elongation of the sulfonamide (**6b**-**6d**) led to an increase of Cyp11 inhibition relative to that of (2S, 4R)-Ketoconazole (**4**). The larger methoxyethyl (**6e**) analog and the 4-tetrahydropyranyl analog (**6f**), on the other hand, are nearly equipotent with the initial lead compound (**4**). Electron withdrawing groups (**6g**-**6j**), 2-thiophene (**6k**), and N-methyl-2-imidazole (**6l**) substitution on the sulfonamide also led to increased Cyp11 potency. Interestingly, **6l** is nearly equipotent at Cy11B1 and Cyp17 (17 nM versus 15 nM).

Table 4: Guinea pig *in vivo* pharmacokinetic profiles of exemplary (2S, 4R)-Ketoconazole analogs.

(n = 3)	**6b**		**6c**		**6d**		**6i**	
Dose(mg/kg)	1	10	1	10	1	10	1	10
Delivery*	IV	PO	IV	PO	IV	PO	IV	PO
Cmax(ng/mL)	446	710	534	358	514	1556	2091	1967
Tmax (h)	0.1	1.17	0.19	0.67	0.08	1.5	0.08	0.67
$t_{1/2}$ (h)	1.7	3.1	1.1	2.2	1.4	2.9	2.0	2.5
Vd (L/kg)	4.4		3.0		2.9		2.5	
Cl (mL/min/kg)	30.5		31		23.8		14.3	
AUC0-inf (ng·h/mL)	560	3714	547	571	715	7576	1165	4599
%F		61.4		10.4		98.5		39.5

*IV vehicle: 20%DMA, 40%TEG, 40%Water. PO vehicle: 98% HPMC (1% in water), 2% Tween80.

Interestingly, Cyp21 potency was significantly increased relative to (2S, 4R)-Ketoconazole (**4**) in all of the sulfonamides examined. While the methyl sulfonamide analog (**6a**) increased Cyp21 activity by a factor of just over 2.6x, larger substituents produced more dramatic results. The addition of a single additional carbon (**6b**), for example, led to an increase in Cyp21 potency of >15 fold (271 nM). This compound is also notable, as its Cyp17/Cyp21 selectivity is the highest observed in the series (>370x) as a result of its high potency at Cyp17 (0.73 nM). The most potent Cyp21 inhibitor identified to date in this series, **6l** (8.0 nM), is also noteworthy. Not only is it >530 fold more potent at Cyp21, it is also nearly equipotent at Cyp17 and Cyp11B1, and it is inactive at Cyp19 (10,000 nM). Selectivity with respect to Cyp19 is an important aspect of potential therapeutic agents, as compounds that are potent inhibitors of Cyp19 (also known as Aromatase) may interfere with estrogen synthesis.[19] The majority of compounds examined were less potent at Cyp19 than the original lead compound (**4**). In some cases (**6e**, **6k**, and **6l**), Cyp19 is completely eliminated. Only two compounds, the methyl sulfonamide analog (**6a**) and the trifluoromethyl sulfonamide analog (**6g**), had Cyp19 IC_{50}s below 1000 nM.

Having identified a series of interesting inhibitors of enzymes critical to cortisol synthesis, we next turned our attention to an evaluation of their pharmacokinetic properties. Given the chronic nature of MetS, we felt that oral delivery would be critical to successful therapeutic intervention. In an effort to identify compounds capable of oral delivery, we first determined the metabolic half-life ($t_{1/2}$) of our compounds in guinea pig liver microsomes (GPLM, table 3). The guinea pig is an established model of metabolic syndrome and methods for cortisol measurement in this species have been previously reported.[20] As indicated in table 3, GPLM stability was moderate (**6f**, $t_{1/2}$ = 15 min) to high (6e, $t_{1/2}$ = 56 min), which suggested that this series of compounds may have sufficient metabolic stability for therapeutic application. In addition, calculated properties (cLogP and TPSA) are suggestive of good permeability, with the notable exception of **6k**, whose cLogP of 6.1 is outside of the desired range (typically 1-5).[21] It is worth noting, however, that even though (±)-Ketoconazole's cLogP is also slightly higher than the desired range (cLogP = 5.2), it is a successful, orally active therapeutic. This suggests that there may be some degree of latitude in the acceptable cLogP values within this class of compounds.

Next, a small set of compounds (**6b**, **6c**, **6d**, and **6i**) was selected for *in vivo* pharmacokinetic evaluation in guinea pigs. Age matched, male Hartley guinea pigs were given a single dose of a test compound via both IV (1 mg/kg) and PO (10 mg/kg) and plasma samples were acquired in order to measure standard PK parameters. As indicated in table 4, small structural changes in the sulfonamide had a significant impact on guinea pig pharmacokinetic properties. The ethyl sulfonamide (**6b**), for example, is moderately bioavailable (61.4%) and has an oral *in vivo* half-life of 3.1 hours, but the addition of a single methyl group (the isopropyl sulfonamide, **6c**) leads to a substantial loss in oral bioavailability (10.4%) and decreased oral *in vivo* half-life ($t_{1/2}$ = 2.2 h). In contrast, the corresponding cyclopropyl sulfonamide (**6d**) is highly bioavailable (98.5%) and its PO half-life ($t_{1/2}$ = 2.9 h) is similar to that observed for **6b**. This result was unexpected, as *in vitro* GPLM data (table 3) had suggested that **6b** would be more metabolically stable than **6c**. Similarly, installation of the electron withdrawing cyano group (**6i**) led to a decrease in oral half-life (2.5 h) and oral bioavailability (39.5%), despite its high stability in GPLM. This suggests that factors other than phase 1 metabolism (e.g. protein binding, phase 2 metabolism) may be playing a significant role in the pharmacokinetic profile of this class of compounds.

In summary, we have identified a novel series of sulfonamide analogs of (2S, 4R)-Ketoconazole that are capable of inhibiting key enzymes in the cortisol synthetic pathway. Exemplary compounds examined in this series possess *in vivo* pharmacokinetic properties necessary for *in vivo* efficacy studies in guinea pig models of MetS to determine their potential for therapeutic utility. The results of these studies are pending.

ACKNOWLEDGMENT

We would like to thank the dean of Temple University School of Pharmacy, Peter H Doukas, for his support and encouragement in pursuing this program. We would also like to the administrative staff of Temple University School of Pharmacy for their assistance. Funding for this program was

provided by Strongbridge Biopharma plc, 900 Northbrook Drive, Suite 200, Trevose, PA 19053 United States. Prior to September 4th, 2015, Strongbridge Biopharma plc was known as Coretendo plc.

References and notes

1. Reaven, G. M. Role of insulin resistance in human disease, *Diabetes*, **1988**, 37, 1595–1607.
2. Anagnostis, P.; Athyros, V. G.; Tziomalos, K.; Karagiannis, A.; Dimitri P. Mikhailidis, D. P. The Pathogenetic role of cortisol in the Metabolic Syndrome: A hypothesis, *J. Clin. Endocrinol. Metab*. **2009** 94, 8, 2692-2701.
3. Welles, B. Glucocorticoids in type 2 diabetes mellitus and the metabolic syndrome, *Drug Dev. Res*. **2006**, 67, 570–573.
4. Pasquali, R.; Vicennati, V.; Cacciari, M.; Pagotto, U. The hypothalamic-pituitary-adrenal axis activity in obesity and the metabolic syndrome, Ann. *N.Y. Acad. Sci*. **2006**, 1083, 111–128.
5. (a) Hakki, T.; Bernhardt, R. CYP17- and CYP11B-dependent steroid hydroxylases as drug development targets, Pharm. Thera. 2006, 111, 1, 27-52. (b) Bureik, M.; Lisurek, M.; Bernhardt, R. The human steroid hydroxylases CYP11B1 and CYP11B2, Bio. Chem. 2002, 383, 10, 1537-1551.
6. White, P. C. Steroid 21-hydroxylase, *Modern Gen*. **2002**, 6, 145-177.
7. (a) Stefanachi, A.; Hanke, N.; Hartmann, R. W.; Pisani, L.; Leonetti, F.; Nicolotti, O.; Catt, M.; Cellamar, S.; Carotti, A. Discovery of new 7-substituted-4-imidazolylmethyl coumarins and 4'-substituted-2-imidazolyl acetophenones open analogues as potent and selective inhibitors of steroid-11β-hydroxylase *European Journal of Medicinal Chemistry*, **2015**, 89, 106-114. (b) Hille, U. E.; Zimmer, C.; Vock, C. A.; Hartmann, R. W. First Selective CYP11B1 Inhibitors for the Treatment of Cortisol-Dependent Diseases, *ACS Medicinal Chemistry Letters*, **2011**, 2, 1, 2-6. (c) Bertagna, X.; Pivonello, R.; Fleseriu, M.; Zhang, Y.; Robinson, P.; Taylor, A.; Watson, C. E.; Maldonado, M.; Hamrahian, A.H.; Boscaro, M.; Biller, B.M. LCI699, a potent 11b-hydroxylase inhibitor, normalizes urinary cortisol in patients with Cushing's disease: results from a multicenter, proof-of-concept study, *J. Clin.Endocrinol. Metab*. **2014**, 99, 4, 1375-1383.
8. de Montellano, P. R. O. Cytochrome P450: structure, mechanism, and biochemistry, 3rd ed. **2005**, Kluwer Aca-demic/Plenum Publishers, New York, New York.
9. (a) Heeres, J.; Backx, L. J. J. ; Mostmans, J. H. Novel 1-(1,3-dioxolan-2-ylmethyl)-1H-imidazoles and 1H-1,2,4-triazoles useful as antifungal and antibacterial agents. US 4144346, **1979**. (b) Levine, H. B.; Cobb, J. M. Oral therapy for experimental coccidioidomycosis with R41 400 (ketoconazole), a new imidazole, The American Review of Respiratory Disease, 1978, 118, 4, 715-721.
10. Van Cauteren, H.; Heykants, J.; De Coster, R.; Cauwenbergh, G. Itraconazole: pharmacologic studies in animals and humans, *Reviews of Infectious Diseases*, **1987**, 9, S 1, S43-S46.
11. Galgiani, J. N. Fluconazole, a new antifungal agent, *Annals of internal medicine*, **1990**, 113, 3, 177-179.
12. Loose, D. S.; Kan, P. B.; Hirst, M. A.; Marcus, R. A. Feldman D "Ketoconazole blocks adrenal steroidogenesis by inhibiting cytochrome P450-dependent enzymes". *J. Clin. Inves*t. **1983**, 71, 5, 1495–1499.
13. Nizoral® tablet package insert, Janssen Pharmaceuticals.
14. Kühn-Velten, W. N.; Lessmann, M. Ketoconazole inhibition of the bifunctional cytochrome P450c17 does not affect androgen formation from the endogenous lyase substrate: The catalytic site remains refractory in the course of intermediary hydroxyprogesterone processing. Biochemical Pharmacology, 1992, 44, 12, 2371–2378.
15. Marin, P.; Birketvedt, G. S. Cortisol regulation in the metabolic syndrome. A novel therapeutic approach. *Immunol. Endocr. Metab. Agents Med. Chem*. **2010**, 10, 2, 76-83.
16. Marin, P. Methods and compositions for treating diabetes, metabolic syndrome and other conditions, WO 2006072881A1, **2006**.
17. Clinical trial NCT01838551, Treatment for Endogenous Cushing's Syndrome, phase 3, **2013**.
18. (a) Patil, S. Synthesis of Ketoconazole impurity by novel method Journal of Chemical and Pharmaceutical Research, 2010, 2, 3, 117-119. (b) Fu, J.; Chen, W. Method for preparing antifungal imidazole derivatives, CN101993436, 2011. (c) Konter, J.; Moellmann, U.; Lehmann, J. NO-donors. Part 17: Synthesis and antimicrobial activity of novel ketoconazole-NO-donor hybrid compounds, Bioorganic and Medicinal Chemistry, 2008, 16, 17, 8294-8300.
19. Simpson, E. R.; Clyne, C.; Rubin, G.; Boon, W. C.; Robertson, K.; Britt, K.; Speed, C.; Jones, M. Aromatase--a brief overview. Annual Review of Physiology, 2002, 64, 93-127.
20. (a) Caillier, B.; Pilote, S.; Patoine, D.; Levac, X.; Couture, C.; Daleau, P.; Simard, .; Drolet, B. Metabolic syndrome potentiates the cardiac action potential-prolonging action of drugs: A possible 'anti-proarrhythmic' role for amlodipine. Pharmacological Research, 2012, 65, 3, 320-327. (b) Patoine, D.; Levac, X.; Pilote, S.; Drolet, B.; Simard, C. Decreased CYP3A expression and activity in guinea pig models of diet-induced metabolic syndrome: is fatty liver infiltration involved? Drug Metabolism and Disposition, 2013, 41, 5, 952-957.
21. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. (March 2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews, 2001, 46, 1-3, 3–26.