Design, Synthesis, and in Vivo SAR of a Novel Series of Pyrazolines as Potent Selective Androgen Receptor Modulators

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A novel series of pyrazolines **2** have been designed, synthesized, and evaluated by in vivo screening as tissue-selective androgen receptor modulators (SARMs). Structure−activity relationships (SAR) were investigated at the R^1 to R^6 positions as well as the core pyrazoline ring and the anilide linker. Overall, strong electron-withdrawing groups at the R^1 and R^2 positions and a small group at the R^5 and R^6 position are optimal for AR agonist activity. The (*S*)-isomer of **7c** exhibits more potent AR agonist activity than the corresponding (*R*)-isomer. (*S*)-**7c** exhibited an overall partial androgenic effect but full anabolic effect via oral administration in castrated rats. It demonstrated a noticeable antiandrogenic effect on prostate in intact rats with endogenous testosterone. Thus, (*S*)-**7c** is a tissue-selective nonsteroidal androgen receptor modulator with agonist activity on muscle and mixed agonist and antagonist activity on prostate.

Introduction

Androgens regulate diverse physiological process involving both reproductive and nonreproductive functions. Most of the signaling effects of androgens are mediated through the androgen receptor (AR), a member of the nuclear receptor superfamily of transcription factors. Deficiencies in circulating levels of the natural AR ligands testosterone (T) and dihydrotestosterone (DHT) in hypogonadal men can be compensated for by administration of exogenous androgens.[1] For decades, AR has been a target for drug development focused upon the treatment of pathological conditions arising from abnormal androgen levels or altered target tissue responsiveness, the improvement of physical performance, and the regulation of male fertility. The primary focus for drug design has been the synthesis of chemicals to regulate the transcriptional activity of AR based on the structural, steroidal or nonsteroidal, and functional androgenic, antiandrogenic, or anabolic properties of ligands.[2] Steroidal AR ligands were first developed by modifying the steroidal structure of endogenous androgens. However, a more widely accepted use of androgen therapy has been hampered by the lack of orally active steroidal preparations with good efficacy and particularly a safe profile for steroidal AR ligands.[3] Efforts to identify more receptor- and tissue-selective ligands without steroid-related side effects and toxicities have shifted focus away from steroid structural templates. Progress has been made over the last 5 years in developing nonsteroidal small molecules that could separate androgenic activities from desirable anabolic activities with improved oral bioavailability and less hepatic toxicity.[4] Whereas nonsteroidal antiandrogens have been used clinically for many years, as exemplified by flutamide and bicalutamide, used clinically in conjunction with LHRH agonists for the treatment of prostate cancer,[5] nonsteroidal androgens have only recently been conceptualized. A new class of molecules targeting androgen receptors called selective androgen receptor modulators (SARMs) has been developed in response to the successfully clinical proof-of-concept of marketing of novel selective estrogen receptor modulators (SERMs).[6] An ideal SARM has antagonist or weak agonist activity in the prostate (androgenic organ) while presenting strong agonist activity in the muscle and bone (anabolic organ). This profile would allow the molecules to treat muscle-wasting conditions, hypogonadism, or age-related frailty while preventing potential risks for nascent or undetected prostrate cancer. AR is widely distributed in tissues such as the prostate, seminal vesicle, male and female genitalia, skin, testis, ovary, cartilage, sebaceous glands, hair follicles, sweat glands, cardiac muscle, smooth muscle, gastrointestinal vesicular cells, thyroid follicular cells, adrenal cortex, liver, pineal, and brain.[7] Tissue selectivity is dependent on the regulation of AR expression, differential DNA binding at the promotor of regulated genes, and tissue-specific protein−protein interactions.[8] In addition, cross-talk with other tissue-specific signaling compounds, nongenomic effects, and heterodimerization with other receptors may contribute to tissue selectivity.[9] Recent progress in this area has already resulted in the design of molecules that display preferential tissue-specific effects and differential activity in androgenic tissues versus anabolic tissues. To date, several chemical scaffolds of nonsteroidal androgens have been identified as SARMs, such as aryl propionamides,[10] bicyclic hydantoins,[11] quinolinones[12] and tetrahydroquinolones.[13] These nonsteroidal molecules behaved as partial agonists in the prostate but full agonists in the *levator ani* muscle as indicated by the castrated rat model. Currently, GTx are planning a Phase II trial of ostarine for use in male burn patients.[14] No SARMs have been advanced into late-stage clinical trials.

Our design approach was to mimic the aryl propionamide SARM structure **1** by incorporating a novel pyrazoline moiety shown in structure **2** (Figure 1). The pyrazoline structure of **2** can be viewed as a locked conformation of **1** by mimicking the internal hydrogen bonding between the *tert*-carbinol and phenoxyl oxygen atom. Our primary goals for developing novel

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[a] Abbreviations: ADME, adsorption−distribution−metabolism−elimination; AR, androgen receptor; DCM, dichloromethane; DHT, dihydrotestosterone; DIPEA, diisopropylethylamine; DMA, dimethylacetamide; DMF, dimethylformamide; DNA, deoxyribonucleic acid; ED_{50}, dose that results in 50% of the maximum effect; HPBCD, hydroxypropyl-β-D-cyclodextrin; IPA, isopropyl alcohol; LHRH, luteinizing hormone-releasing hormone; NCS, *N*-chlorosuccinimide; SAR, structure−activity relationship; SARM, selective androgen receptor modulator; SD, standard deviation; SEM, standard error of the mean; SERM, selective estrogen receptor modulator; T, testosterone; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TP, testosterone propionate

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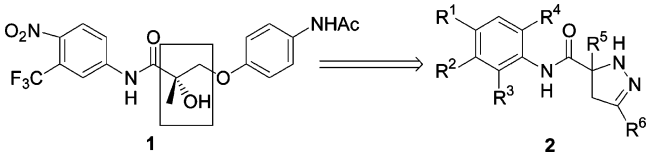


Figure 1.

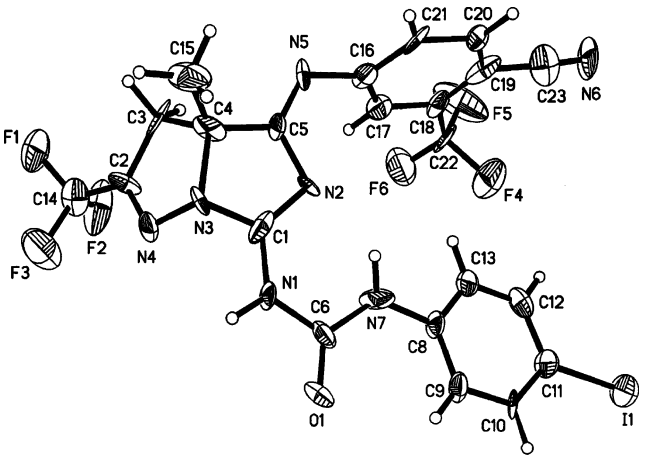


Figure 2. X-ray crystallographic structure of (*R*)-**13**.

SARMs are shown below. First, fusing of the phenoxymethyl *tert*-carbinol group of **1** into a five-membered heterocyclic ring generated the pyrazoline core structure. Second, replacement of the potentially problematic 4-nitro group with a structurally and metabolically favorable substitution should provide more advantage for toxicity and tolerability profile. Finally, we are aiming at reducing the molecular weight by replacing the *p*-acetamidophenyl group with a small side chain for a better pharmacokinetic property.

Chemistry

The synthetic route to compounds **5**, **6**, **7**, and **8** was reported in our early paper.[15] The pyrazolines described in this report were prepared primarily by the method shown in Scheme 1. Therefore, reaction of the appropriately substituted acrylamides **5** with various tosylhydrazones under the treatment with NaH through 1,3-dipolar cycloaddition afforded the desired pyrazolines **6a**−**i**, **7a**−**s**, **8a**−**g** and their isomers **11**, which could be converted to pyrazolines through acid-catalyzed isomerization. The corresponding acrylamides **5** were prepared by direct coupling of anilines **3** to 2-alkyl acrylic acids **4** using the standard thionyl chloride or oxalyl chloride method. Anilines **3** were obtained from commercial sources or were prepared through multistep chemical transformations described in the Supporting Information. The synthesis of **9c**, **9d**, **9e**, and **9f** is outlined in Scheme 2. 1,3-Dipolar cycloadditions of **5b** with the corresponding carbohydrazonoyl chloride in the presence of triethylamine (dipoles formed in situ) generated the cycloadducts **9c** and **9d** in reasonable yields. Compounds **9e** and **9f** were synthesized in the same manner using the oximes as the dipole precursors (dipole formed under oxidative condition). All these reactions were highly regioselective that only 3,4-dihydro-*2H*-pyrazole structures were isolated as the only products without detecting any isomers. Compound **7c** was treated with MeOTf to give **9a** where methylation occurred at the pyrazoline ring. The trifluoromethylacetamide analogue **9g** was prepared by reaction of **7c** with trifluoroacetyl anhydride in the presence of triethylamine (Scheme 3). Ethylated compounds **9b** and **10b** were isolated from the reaction of **7c** with $Et_3O^+BF_4^-$ in the buffer solution. Interestingly, **10b** was obtained as a pair of diastereomers with the structure similar to the [4,5]-pyrazoline **11**. Because of the acid-catalyzed isomerization process of **11** to **7**, we decided to carry over **10b** directly into biological evaluation without transforming it into the [3,4]-pyrazoline isomer. Thioanilide **10c** was obtained by treatment of **7c** with Lawesson's reagent. Compound **10c** was converted to **10a** through alkylation of the thioanilide followed by displacement with MeOH (Scheme 4). Determination of the absolute stereochemistry of enantiomeric **7c** was shown in Scheme 5. The racemic **7c** was subjected to chiral separation by ChiralPak AD HPLC column to afford (−)-**7c** and (+)-**7c**. Preparation of (−)-**12** from (+)-**7c** has been reported in our recent communication.[15] Compound **7c** was transformed into its thioamide analogue by treatment with Lawesson's reagent, followed by alkylation of the thioamide with EtI in the presence of K_2CO_3 to afford the corresponding thioimidate, which was converted into the bicyclic imidazolopyrazole derivative (−)-**12** by reaction with NH_2CN in the presence of K_2CO_3 through an intramolecular 5-exo cyclization. The enantiomeric urea (−)-**13** bearing a heavy atom derived from the enantiomeric (−)-**12** gave crystals suitable for X-ray crystallographic analysis. The structure of a crystal of the (−)-**13** enantiomer was determined to have the (3*R*) stereochemistry (Figure 2), which suggested that its precursor (+)-**7c** should bear the same (3*R*) stereochemistry.

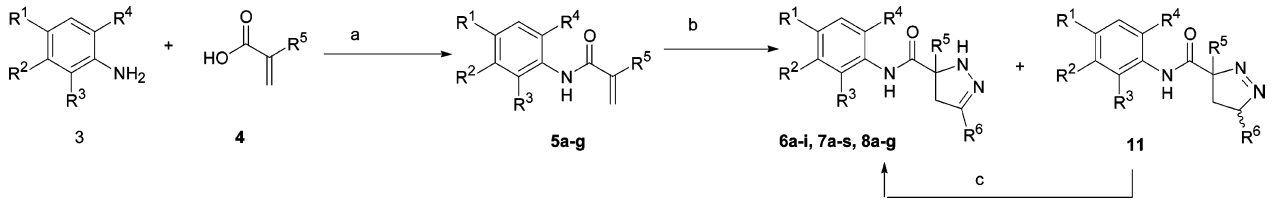
Results and Discussions

Initially we proceeded with several in vitro assays as our primary guide for screening. However, we observed that the AR in vitro assay could not always predict the in vivo tissue-selective pharmacologic activity. A similar observation was reported in the literature.[16] Moreover, no correlations between AR binding affinity, AR-mediated transcriptional activation, and in vivo pharmacologic activity were found in our initial investigation on this series of compounds. We decided to use a modified Herschberger assay[17] as an alternative in vivo screening tool to evaluate our analogues. This in vivo protocol provided us a substantial timesavings because it did not require in vitro binding assays, functional assays, and in vivo ADME screening before moving to an oral efficacy model. This model normally took only about 40 mg of compounds and provided us screening results in less than a week.

All compounds were tested in immature castrated male Sprague Dawley rats (approximately 50 g, Charles River) agonist and antagonist assays. In these studies, the weights of the ventral prostate were used as the indicators of androgenic activity, while the weight of *levator ani* muscle was used as the indicator of anabolic activity. When using such a screening protocol, it is important to bear in mind that the results are a combination of a test compound's ADME properties and intrinsic efficacy. To mitigate differences in the test compounds' rate of dissolution, we selected 20% hydroxyl propyl β-cyclodextrin (HPBCD) as our oral dosing vehicle, as it almost dissolved all compounds in the series.

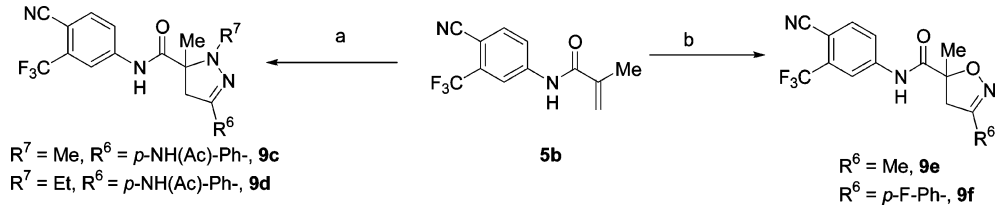
The compounds identified from immature rat screening will be further tested in castrated mature male rats (150 to 450 g) for their abilities to stimulate prostate and *levator ani* muscle weights in the absence of endogenous testosterone. This model is identical to the castrated immature rat model described above, except for the age of the animals. It is our experience that effects in mature animals are more predictive of androgen receptor pharmacology than effects in immature animals. Once androgen agonist activity is confirmed, the compounds will be tested in intact mature male rats for its ability to reduce prostate weight

Scheme 1[a]



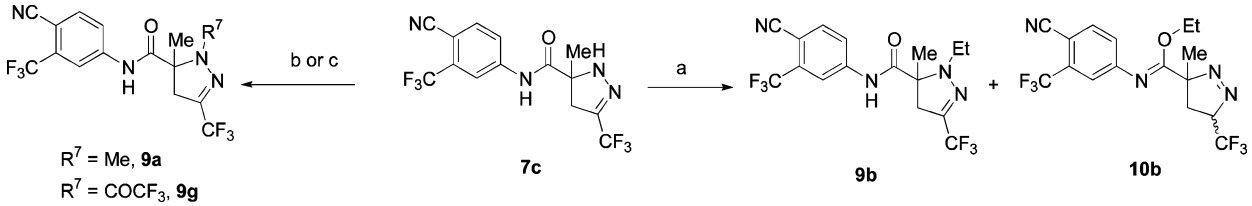
[a] (a) $SOCl_2$, DMA, 0 °C or $(COCl)_2$, DMF (cat.), TEA, DCM, 0 °C to r.t.; (b) $R^6CH{=}N$-NHTs, NaH, THF, 0 °C to reflux; (c) TFA, DCM, rt.

Scheme 2[a]



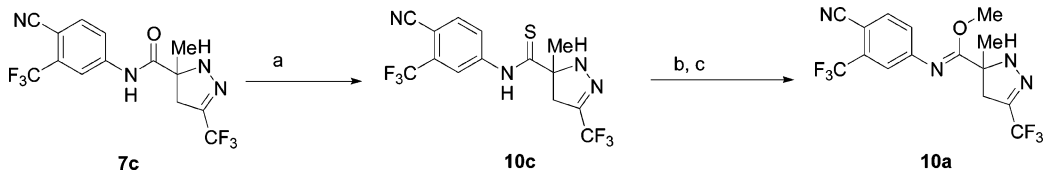
[a] (a) (1) $R^7NH{-}N{=}CH{-}R^6$, NCS, Me_2S, (2) TEA, THF, 0 °C to rt; (b) $HO{-}N{=}R^6$, NaOCl, DCM, 0 °C to r.t.

Scheme 3[a]



[a] (a) $Et_3O^+BF_4^-$, Na_2HPO_4, DCM, 0 °C to r.t.; (b) for **9a**, MeOTf, DIPEA, DCM 0 °C to r.t.; (c) for **9g**, $(CF_3CO)_2O$, TEA, DCM, 0 °C to r.t.

Scheme 4[a]



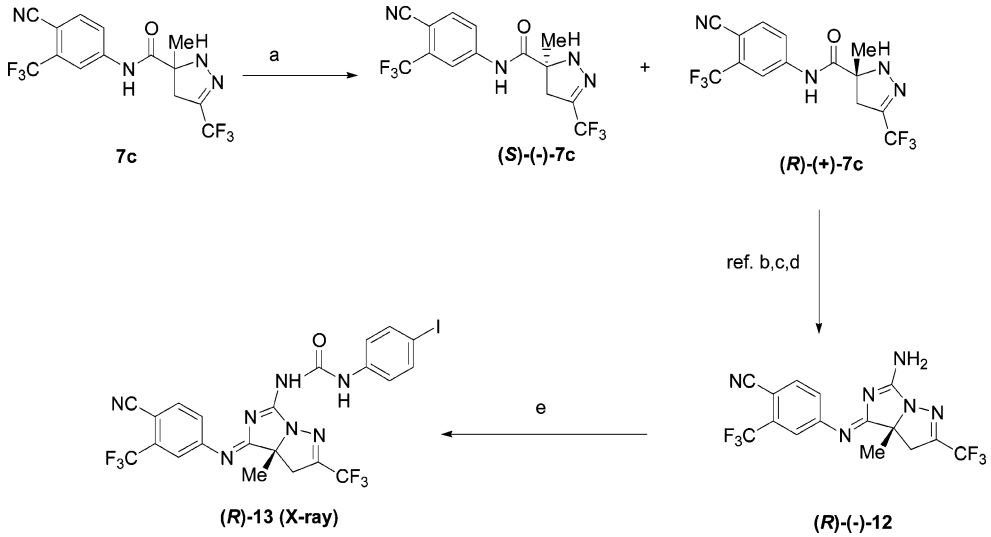
[a] (a) Lawesson's reagent, toluene, 120 °C; (b) K_2CO_3, EtI, DMF, 50 °C; (c) MeOH, sealed tube, reflux.

in the presence of endogenous testosterone. Effects on the weights of *levator ani* are also measured.

We focused our initial efforts by examining the SAR of the anilide R^1 and R^2 groups as well as R^6 as aryl groups. Previous substitution studies on this region of structure **1** series revealed an extremely narrow structural requirement at this position.[18] Thus, R^1 were selected from electron-deficient groups such as nitro, cyano, and halogen along with electron-deficient and lipophilic R^2 groups such as trifluoromethyl and cyano. The pyrazoline analogue (**6a**) of compound **1** was prepared for direct comparison on this new scaffold. As shown in Table 1, **6a** demonstrated AR agonist activities in the castrated immature rats model. It prevented castration, caused tissue weight loss, and behaved as a partial agonist in both the prostate (15% stimulation of the prostate weight at 2 mg/d) and the *levator ani* (35% stimulation of the *levator ani* weight at 2 mg/d). Although only weak agonist activity was observed for **6a**, it served as a starting point for further optimization. The R^6 substitution of **6a** could be extended to *p*-trifluoroacetamidophenyl (**6c**) and *p*-fluorophenyl group (**6i**) without loss of the efficacy. This finding is consistent with similar observations with structures **1** series. Due to the potential toxicity of nitro group, alternative substitutions were also pursued for better safety profile. Interestingly, the cyano group (**6b**) is an efficient replacement of the nitro group with improved efficacy (57% stimulation of the *levator ani* weight at 2 mg/d), which is not parallel to the SAR of structure **1** series. Replacement of cyano or nitro group on the phenyl ring with chlorine (**6e**) resulted in dramatic loss in efficacy. Installation of a cyano group at R^2 position (**6d**) was also detrimental to the efficacy. As for R^6, the *p*-acetamidophenyl group could be replaced with *p*-fluorophenyl (**6h**) or *p*-acetamidobenzyl (**6g**) group without loss of the efficacy but replacement with *m*-acetamidophenyl (**6f**) abolished the activity. The data in Table 1 clearly indicated that both the substitutions at R^1 or R^2 positions of the phenyl rings and the substitution at R^6 position contributed to the overall activities of this series of compounds. According to the primary screening result, **6b** was chosen to advance into the efficacy model - mature castrated rat agonist assay. However, in this 2-week test, we found that **6b** was not chemically stable enough in the vehicle used. This was most likely due to the ring strain on the pyrazoline ring with the bulky aryl substitution at R^6 position, which could gradually lead to thermal isomerization of **6b** to its isomer structure **11**. Followed by N_2 extrusion, **6b** could partially decompose into unidentified mixture.

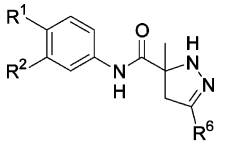
Our attention turned next to the modification on the R^6 position by incorporating a small alkyl instead of bulky aryl group for more chemically stable analogues and better oral bioavailability. Meanwhile, further SAR around R^1, R^2, and R^5 was built up through in vivo screening. The effect on in vivo activity of alterations made to R^1, R^2, R^5, and R^6 of the pyrazoline scaffold is summarized in Table 2. While removal

Scheme 5[a]



[a] (a) ChiralPak AD column, heptane:IPA; (b) Lawesson's reagent, toluene, 120 °C; (c) K_2CO_3, EtI, DMF, 50 °C; (d) NH_2CN, K_2CO_3, dioxane, 80 °C; (e) *p*-iodo-phenyl isocyanate, THF, 50 °C.

Table 1. SAR on the Pyrazoline Structure (R^1, R^2, and R^6)

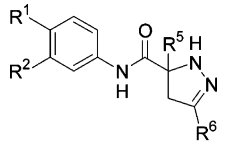


compd[a]	R^1	R^2	R^6	prostate stimulation (%)	*levator ani* stimulation (%)
TP[b,c]				100	100
6a	NO_2	CF_3	*p*- NH(Ac)-Ph-	15	35
6b	CN	CF_3	*p*-NH(Ac)-Ph-	16	57
6c	NO_2	CF_3	*p*-NH($COCF_3$)-Ph-	10	21
6d	CN	CN	*p*-NH(Ac)-Ph-	<10	<10
6e	Cl	CF_3	*p*-NH(Ac)-Ph-	<10	17
6f	CN	CF_3	*m*- NH(Ac)-Ph-	<10	<10
6g	CN	CF_3	*p*-NH(Ac)-Bn-	24	51
6h	CN	CF_3	*p*-F-Ph-	20	44
6i	NO_2	CF_3	*p*-F-Ph-	<10	18

[a] All compounds were administered via p.o. (vehicle: 20% cyclodextrin) once daily at a dose rated of 2 mg/day for 5 days. The data was normalized to control group administrated with vehicle ($n = 3$/group) and adjusted to body weight. [b] Testosterone propionate was administered subcutaneously by injection at the nape of the neck at 1 mg/d, in a volume of 0.1 mL in sesame oil. [c] Set up as 100% stimulation.

of the substitution at the R^6 position (**7a**) was detrimental to the androgen agonist activity on both prostate and *levator ani* muscle, substitution at the R^6 position with a methyl group (**7b**) could bring back the strong agonist efficacy with 62% stimulation on *levator ani* and 29% stimulation on prostate. Compound **7b** bearing a metabolically favorable trifluoromethyl group at the R^6 position was even slightly more potent with 71% stimulation on *levator ani*. Both **7b** and **7c** were roughly equipotent to the lead structure **6b** in Table 1, which demonstrated a successful replacement of the *p*-acetamidophenyl group at **6b**. Similar to what was observed in the investigation at R^1 and R^2 positions in Table 1, both nitro (**7d**) and halogen substitutions (**7e**, **7f**) at the R^1 position significantly attenuated the efficacy. Evaluation of the R^2-substituted analogues revealed that this was the sensitive area for activity where replacement of trifluoromethyl with chloro group or removal trifluoromethyl abolished the efficacy. Larger R^6 substitutions were not tolerated for efficacy even for a slightly bigger ethyl group with only just one carbon extension (**7i**, **7k**, **7l**). Compound **7j** bearing

Table 2. Further SAR on the Pyrazoline Structure (R^1, R^2, R^5 and R^6)

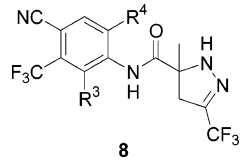


compd[a]	R^1	R^2	R^5	R^6	prostate stimulation (%)	*levator ani* stimulation (%)
TP[b,c]					100	100
7a	CN	CF_3	Me	H	<10	<10
7b	CN	CF_3	Me	Me	29	62
7c	CN	CF_3	Me	CF_3	28	71
7d	NO_2	CF_3	Me	CF_3	38	21
7e	Cl	CF_3	Me	CF_3	<10	15
7f	Br	CF_3	Me	CF_3	12	41
7g	Cl	Cl	Me	CF_3	10	<10
7h	CN	H	Me	CF_3	<10	<10
7i	CN	CF_3	Me	Et	<10	<10
7j	CN	CF_3	Me	*i*-Pr	<10	<10
7k	CN	CF_3	Me	CH_2CF_3	<10	10
7l	CN	CF_3	Me	CF_2CF_3	<10	15
7m	CN	CF_3	CF_3	CF_3	13	37
7n	CN	CF_3	CF_3	Me	46	64
7o	NO_2	CF_3	Me	CO_2Et	<10	<10
7p	CN	CF_3	Et	CF_3	24	67
7q	CN	CF_3	*n*-Pr	CF_3	<10	18
7r	CN	CF_3	Me	*n*-Pr	<10	<10
7s	CN	CF_3	Me	*t*-Bu	<10	36

[a] All compounds were administered via p.o. (vehicle: 20% cyclodextrin) once daily at a dose rated of 2 mg/day for 5 days. The data was normalized to control group administrated with vehicle ($n = 3$/group) and adjusted to body weight. [b] Testosterone propionate was administered subcutaneously by injection at the nape of the neck at 1 mg/d, in a volume of 0.1 mL in sesame oil. [c] Set as 100% stimulation.

isopropyl, **7r** bearing *n*-propyl, and **7o** bearing ethyl carboxylate substitutions were inactive for androgen agonist activity. The trend did not extend to the *tert*-butyl analogue **7s**, however, which exhibited 36% stimulation on *levator ani*. Further SAR at the R^5 revealed that trifluoromethyl group (**7m** and **7n**) was tolerated for efficacy, and adding a little size such as ethyl group (**7p**) also retained the efficacy, though strong efficacy disappeared for **7q** bearing an *n*-propyl group at the R^5 position, which suggested that the size of R^5 cannot be larger than ethyl. In addition, the effect of adding the third substitution on the

Table 3. SAR on the Pyrazoline Structure (R^3 and R^4)



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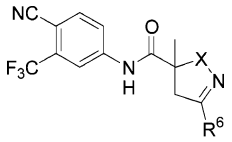
compd[a]	R^3	R^4	prostate stimulation (%)	*levator ani* stimulation (%)
TP[b,c]			100	100
8a	Et	H	14	88
8b	H	Et	<10	<10
8c	H	OMe	18	52
8d	H	Cl	15	57
8e	H	I	47	86
8f	H	CN	<10	<10
8g	H	SEt	<10	42

[a] All compounds were administered via p.o. (vehicle: 20% cyclodextrin) once daily at a dose rated of 2 mg/day for 5 days. The data was normalized to control group administrated with vehicle ($n = 3$/group) and adjusted to body weight. [b] Testosterone propionate was administered subcutaneously by injection at the nape of the neck at 1 mg/d, in a volume of 0.1 mL in sesame oil. [c] Set as 100% stimulation.

anilide aromatic ring was examined (Table 3). Introduction of an additional ethyl group at the R^3 position (**8a**) resulted in the same efficacy as that of **7c** on *levator ani*, while the analogue bearing an ethyl group at the R^4 position (**8b**) did not present clear agonist efficacy. As the data indicated, the various substitutions at the R^4 position resulted in quite different outcomes for the androgen agonist activity. Moderate to strong stimulations on *levator ani* were observed with some R^4-substituted analogues, such as the methoxy (**8c**) and ethylthio (**8g**) analogues with partial agonist activity and halogen-substituted analogues **8d** and **8e** with full agonist activity. However, addition of a cyano group at the R^4 position (**8f**) diminished the efficacy. Some general observations can be made from the SAR studies on R^1-R^6 shown in Tables 1 and 2. (1) The pyrazoline core is the effective mimic of the *tert*-carbinol side chain of structure **1**. (2) Small alkyl groups can replace the *p*-acetamidophenyl group. Methyl and trifluoromethyl appear to be the best replacements. (3) A nitro group can be replaced by a cyano group at the R^1 position. (4) Small alkyl groups at the R^5 position are preferred. Modification of the core five-membered heterocyclic ring is illustrated in Table 4. Masking of the pyrazoline ring of **7c** or **6b** with methyl group (**9a**, **9c**) resulted in partial or total loss of the agonist activity, while the ethylated (**9b**, **9d**) and the trifluoromethylacylated (**9g**) analogues were somehow tolerated for efficacy showing corresponding 95%, 55%, and 70% stimulations on *levator ani*. Replacement of the pyrazoline ring with the dihydro-isoxazole core resulted in total loss of activity (**9e**, **9f**). The anilide functionality was briefly studied, as shown in Table 5. In this area, we previously reported the SAR study on the amidate analogues for replacement of the anilide moiety of **7c**.[15] Here, imidate and thioanilide analogues were prepared and tested in the screening assay. While methyl and ethyl imidates (**10a**, **10b**) maintained the efficacy, the thioanilide **10c** was found toxic and lethal to the testing animals.

Selected compounds were prepared in enantiomeric pure forms for testing in castrated immature male rats agonist and antagonist assays (Table 6). Dose−response curves and EC_{50} values were generated for potent compounds, and the data was summarized in Table 6. Compound (*S*)-**7c** presented dose-dependent pharmacological responses in both anabolic (*levator ani*) and androgenic (prostate) tissues (Figure 3). In the absence

Table 4. Modification on the Pyrazoline Structure



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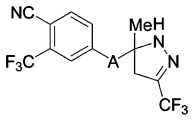
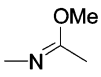
compd[a]	X	R^6	prostate stimulation (%)	*levator ani* stimulation (%)
TP[b,c]			100	100
9a	*N*-Me	CF_3	<10	44
9b	*N*-Et	CF_3	47	95
9c	*N*-Me	*p*-NH(Ac)-Ph-	<10	<10
9d	*N*-Et	Me	18	55
9e	O	Me	<10	<10
9f	O	*p*-F-Ph-	<10	<10
9g	*N*-$COCF_3$	CF_3	30	70

[a] All compounds were administered via p.o. (vehicle: 20% cyclodextrin) once daily at a dose rated of 2 mg/day for 5 days. The data was normalized to control group administrated with vehicle ($n = 3$/group), adjusted to body weight. [b] Testosterone propionate was administered subcutaneously by injection at the nape of the neck at 1 mg/d, in a volume of 0.1 mL in sesame oil. [c] Set as 100% stimulation.

Table 5. Modification on the Anilide Moiety



10

Compounds[a]	A	Prostate stimulation (%)	Levator ani stimulation (%)
TP[b,c]		100	100
10a		30	86
10b[d]		22	75[e]
10c		-[f]	-[f]

[a] All compounds were administered via p.o. (vehicle: 20% cyclodextrin) once daily at a dose rated of 2 mg/day for 5 days. The data was normalized to control group administrated with vehicle ($n = 3$/group), adjusted to body weight. [b] Testosterone propionate was administered subcutaneously by injection at the nape of the neck at 2 mg/kg, in a volume of 0.1 mL in sesame oil. [c] Set as 100% stimulation. [d] [4,5]-Pyrazoline isomer was formed during the reaction and was tested. [e] ED_{50} *lev. ani* 0.25 mg/d. [f] The compound is lethal to the animals and no data could be obtained.

of testosterone, (*S*)-**7c** was a tissue-selective androgen agonist, with ED_{50} 0.11 mg/d (90% stimulation at 3 mg/d) on *levator ani* and ED_{50} 0.76 mg/d (51% stimulation at 3 mg/d) on prostate. On the other hand, in the presence of exogenous testosterone, the compound at a single dose of 2 mg/day inhibited stimulation of prostate weight by 40%, which exhibited its mixed agonist and antagonist activity on prostate. Interestingly, (*S*)-**7c** acted as the eutomer that contributed the observed in vivo efficacy, while its (*R*)-**7c** was a weaker agonist showing ED_{50} 0.86 mg/

Table 6. Mixed Agonist and Antagonist Activity on Selected Compounds in Immature Rats Model

compd[a]	*levator ani* potency/stimulation[b] ED_{50} (mg/d) (%)	prostate potency/ stimulation[b] ED_{50} (mg/d) (%)	prostate inhibition[c] (%)
TP	0.1	0.1	
bicalutamide[d]			70
(*S*)-**7c**	0.11(90)[e]	0.76 (51)[e]	40
(*R*)-**7c**	0.86(49)[e]	0.94 (29)[e]	22
(*S*)-**9b**	0.12	2.2	18
(*R*)-**9b**	0.45	>3.0	<10
(*S*)-10b	0.86	>3.0	27
(*R*)-**10b**	0.10	0.16	<10

[a] $p < 0.01$ relative to vehicle (one-way ANOVA w/Dunnett's post test), mean ± SD, $n = 3$, tissue weights adjusted to body weight. [b] A four point dose-dependent study at doses of 0.1, 0.3, 1, and 3 mg/d on prostate and *levator ani* weight in castrated immature Sprague Dawley rats. Testosterone treated (1 mg/day) control group is set at 100%. ($n = 3$/group). Tissue weight is adjusted to body weight. [c] Prostate weight inhibition % in testosterone treated castrated immature Sprague Dawley rats. Dose = 2 mg/day. The control group administered with testosterone (0.1 mg/day s.c.) was set to 100%. ($n = 3$/group). Tissue weight is adjusted to body weight. [d] Average value based on 10 tests. [e] Prostate and *levator ani* weight stimulation % in castrated immature Sprague Dawley rats. Dose = 3 mg/day, tissue weights adjusted to body weight.

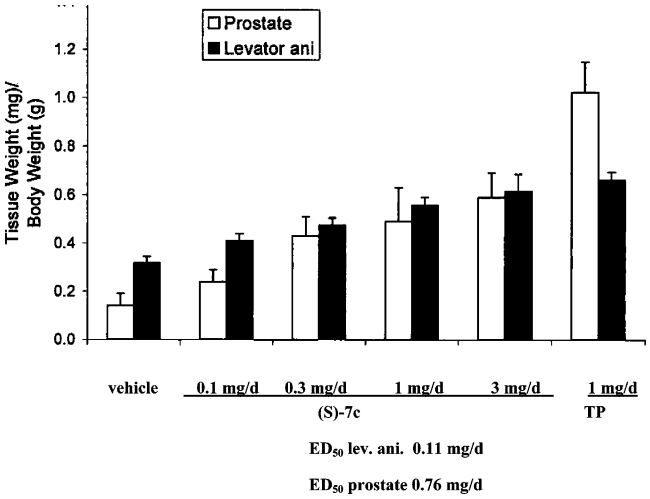


Figure 3. Effect of (*S*)-7c on *levator ani* and prostate weights in orchidectomized immature rats following oral administration ($p < 0.01$ relative to vehicle, one-way ANOVA w/Dunnetts post-test, three rats per group, mean ± SD).

day (49% stimulation at 3 mg/d) on *levator ani* and ED_{50} 0.94 mg/day (29% stimulation at 3 mg/d) on prostate at the same dose in the castrated immature rats agonist assay. For two enantiomers of the pyrazoline ring ethylated analogues (*S*)-**9b** and (*R*)-**9b**, agonist activities on *levator ani* correlated to that of (*S*)-**7c** and (*R*)-**7c**. (*S*)-**9b** stimulated the *levator ani* more efficiently than its enantiomer, (*R*)-**9b** (ED_{50} 0.12 mg/d vs 0.45 mg/d). However, the activities on the *levator ani* showed a stereoselective preference for the *R* isomer, (*R*)-**10b** of the ethyl imidates over the corresponding S isomer, (*S*)-**10b**. It is interesting that the configuration about the chiral center for the eutomer changed from S of the anilide to R of the imidate. The ethyl group of **10b**, which might be engaged in additional AR binding, can result in the difference of configurational preference between **7c** and **10b**. Both (*S*)-**9b** and (*R*)-**10b** inhibited stimulation of prostate weight less effective than (*S*)-**7c** at a single dose of 2 mg/day in the immature male rats antagonist assay.

Selected compounds were advanced for further pharmacological evaluations according to the immature male rats screening result. These compounds were first tested in castrated mature male rats to confirm androgen agonist activity. This was our key efficacy model. As shown in Table 7, **7c** had an ED_{50} on *levator ani* of 7.9 mg/kg and an ED_{50} on prostate of >30 mg/kg (25% stimulation at 30 mg/kg). Both of the ethyl analogues at the R^3 and R^5 positions (**8a**, **7p**) attenuated the AR agonist activity with an ED_{50} on *levator ani* of 21 and 17 mg/kg, respectively. The enantiomers of **7c** were further tested in these animal models. As the eutomer contributing the efficacy, (*S*)-**7c** exhibited an ED_{50} on prostate of >30 mg/kg (46% stimulation at 30 mg/kg) and on *levator ani* of 3.8 mg/kg (120% stimulation at 30 mg/kg). Maximal *levator ani* stimulation (ED_{max}, 100%) was observed at 10 mg/kg. The corresponding enantiomer, (*R*)-**7c**, did not show significant potency on both *levator ani* and prostate. For comparison, TP in a separate experiment had an ED_{50} on prostate of 0.25 mg/kg (maximal 97% stimulation at 1 mg/kg) and on muscle of 0.17 mg/kg (maximal 99% stimulation at 1 mg/kg). Compounds (*S*)-**7c** and (*R*)-**7c** were further tested in intact mature male rats to assess antagonist activity on endogenous testosterone. Compound (*S*)-**7c** reduced prostate weight dose-dependently, with an ED_{50} of >30 mg/kg (33% inhibition at 30 mg/kg). At the same time, there was no significant dose-dependent effect on *levator ani* weight. For comparison, the androgen antagonists flutamide and bicalutamide reduced both prostate and *levator ani* weights by 51 to 67% at 30 mg/kg. These data strongly indicated that (*S*)-**7c** was a tissue-selective nonsteroidal androgen receptor ligand with agonist activity on rat muscle and mixed agonist and antagonist activity on the rat prostate.

Table 7. Evaluations on Selected Compounds in Castrated and Intact Mature Rats Models

compd[a]	*levator ani*. potency/stimulation,[b] ED_{50} (mg/kg) (%)	prostate potency/ stimulation,[b] ED_{50} (mg/kg) (%)	prostate inhibition[c] (%)
TP	0.17	0.25	
flutamide[d]			51
bicalutamide[d]			67
7c	7.9	>30 (25%)	nt[e]
7p	21	>30 (19%)	nt[e]
8a	17	>30 (18%)	nt[e]
(*S*)-**7c**	3.8 (120%)	>30 (46%)	33
(*R*)-**7c**	>30 (9%)	>30 (4%)	25

[a] $p < 0.01$ relative to vehicle (one-way ANOVA w/Dunnett's post test), mean ± SD, $n = 3$, tissue weights adjusted to body weight. [b] The data were obtained in castrated rats model. A five point dose-dependent study at doses of 0.3, 1, 3, 10, and 30 mg/kg. Percentage stimulation at 30 mg/kg is shown. [c] The data were obtained in intact rats model. Percentage inhibition at dose of 30 mg/kg is shown. [d] Average value. [e] nt = not tested.

As complementary studies, in vitro assays were conducted to confirm the molecular mechanism of the efficacious compounds. Compound (*S*)-**7c** was evaluated for its ability to competitively bind to the full-length rat AR in transiently transfected monkey kidney (COS-7) cells.[19] It inhibited binding of the tracer with a K_i of 630 nM. For comparison, bicalutamide as the standard AR anatagonist in this assay had a K_i of 940 nM. Compound (*S*)-**7c** was also evaluated in LNCaP human prostate cancer cells (cell-based functional assay)[20] for its ability to induce secretion of androgen-responsive prostate-specific antigen from the cells. It showed mixed agonist and antagonist activity, with an EC_{50} of 140 nM and an IC_{50} of 14 μM. For comparison, bicalutamide showed antagonist activity with an IC_{50} of 350 nM. These data confirmed the ability of (*S*)-**7c** to modulate transcriptional activity of androgen receptor to induce the observed pharmacologic response. Compound (*S*)-**7c** was evaluated for its ability to bind to other sex steroid receptors in vitro, such as the progesterone receptor B-form (PR), the glucocorticoid receptor (GR), and the estrogen receptors α

(ERα) and β (ERβ) and exhibited no significant cross-reactivity. The detailed pharmacology of (*S*)-**7c** will be reported elsewhere.[21]

Conclusions

A novel series of pyrazolines have been identified as nonsteroidal androgen receptor agonists by means of intuitive design and systematic in vivo SAR studies. The pyrazoline core is demonstrated as an effective substitute for the *tert*-carbinyl side chain of **1**. 4-Nitro group at R^1 position of the aromatic ring can be replaced by 4-nitrile group with improved oral efficacy. Reducing the bulkiness at the R^6 position on the pyrazoline ring by substituting the *p*-acetamidophenyl with a small trifluorormethyl group not only improved the chemical stability but could also generate compounds with better pharmacokinetic profiles. The (*S*)-isomer exhibits stronger potency for AR agonist activity than the corresponding (*R*)-isomer, as exemplified by **7c**. As the result of screening efforts, (*S*)-**7c** was advanced into castrated and intact mature male rats assays. It is demonstrated as a potent oral active tissue-selective nonsteroidal androgen receptor modulator with agonist activity on muscle and mixed agonist and antagonist activity on prostate, which could be employed in male contraception, hypogonadism of aging, osteoporosis, and in the treatment of muscle wasting conditions in cancer, AIDS, and female sexual dysfunction.

Experimental Section

Chemistry. General Procedures. ^{1}H NMR spectra were recorded at 400 MHz or 300 MHz with chloroform-*d* or MeOD-d_4 as solvent on a Brucker AVANCE300 or AVANCE400 spectrometer. Chemical shifts are reported in ppm downfield from TMS as an internal standard. Thin-layer chromatography was carried out using 2.5 × 7.5 cm silica gel 60 (250 μM layer) plates with UV detection. Anhydrous sodium sulfate was employed to dry organic extracts prior to concentration by rotary evaporation. Flash chromatography was usually done using a CombiFlash companion system with prepacked silica gel cartridges purchased from AnaLogix. Solvents from J.T. Baker or Aldrich and all other commercially available reagents were used without further purification. Melting points were taken using a Thomas-Hoover MelTemp apparatus without any correction. Microanalysis was done by Quantitative Technologies Inc., Whitehouse, NJ. Mass spectra were obtained on a Hewlett-Packard 5989A quadrupole mass spectrometer. HPLC analysis was carried on Agilent 1100 Series LC/MSD equipment. High-resolution mass spectra were obtained on M-Scan's VG Analytical ZAB 2SE high field mass spectrometer. X-ray crystal structure determination was measured using graphite-monochromated Mo Kα radiation on a Bruker SMART APEX CCD single-crystal diffraction.

Typical Procedures for Synthesis of Acrylamides 5. The literature procedure was modified to prepare **5** as shown the below.[22]

2-Methyl-*N*-(4-cyano-3-trifluoromethyl-phenyl)-acrylamide 5b. Methyl acrylic acid (510 mg, 6 mmol) in DMA (10 mL) was treated with thionyl chloride (714 mg, 6 mmol) at 0 °C. The mixture was stirred for 30 mi, and then 4-cyano-3-trifluoromethyl-aniline (1.0 g, 6.0 mmol) was added. The resulting suspension was stirred overnight and then quenched with $NaHCO_3$. The reaction mixture was extracted with ethyl acetate, washed with brine, and dried with Na_2SO_4. The resulting concentrated crude product was purified on CombiFlash System with silica gel prepacked cartridge using ethyl acetate and hexanes as eluent to yield the title compound as a yellow solid (1.12 g). Yield, 75%. The spectroscopic data were identical to that reported in the literature.[23]

2-Methyl-*N*-(4-nitro-3-trifluoromethyl-phenyl)-acrylamide 5a. Compound **5a** was prepared from coupling 4-nitro-3-trifluoromethyl-aniline and methyl acrylic acid according to the procedure used to prepare **5b**. Yield, 72%. The spectroscopic data were identical to that reported in the literature.[22]

2-Methyl-*N*-(3,4-di-cyano-phenyl)-acrylamide 5c. Compound **5c** was prepared from coupling 3,4-dicyano-trifluoromethyl-aniline and methyl acrylic acid according to the procedure used to prepare **5b**. Yield, 81%. ^{1}H NMR ($CDCl_3$) δ 8.25 (s, 1H), 8.05 (s, br, 1H), 7.95 (d, J = 7.5 Hz, 1H), 7.74 (d, J = 7.5 Hz, 1H), 5.88 (s, 1H), 5.65 (s, 1H), 2.11 (s, 3H). MS (*m*/*z*): 234, $(M + H)^+$.

2-Methyl-*N*-(4-Chloro-3-trifluoromethyl-phenyl)-acrylamide 5d. Compound **5d** was prepared from coupling 4-chloro-3-trifluoromethyl-aniline and methyl acrylic acid according to the procedure used to prepare **5b**. Yield, 81%. ^{1}H NMR ($CDCl_3$) δ 7.90 (s, 1H), 7.70 (dd, J = 7.5 Hz, 2.0 Hz, 1H), 7.40 (d, J = 7.5 Hz), 5.80 (s, 1H), 5.50 (s, 1H), 2.00 (s, 3H). MS (*m*/*z*): 263, $(M + H)^+$.

2-Methyl-*N*-(4-bromo-3-trifluoromethyl-phenyl)-acrylamide 5e. Compound **5e** was prepared from coupling 4-bromo-3-trifluoromethyl-aniline and methyl acrylic acid according to the procedure used to prepare **5b**. ^{1}H NMR ($CDCl_3$) δ 7.90 (s, 1H), 7.85 (s, br, 1H), 7.69 (d, J = 7.5 Hz, 1H), 7.61 (d, J = 7.5 Hz, 1H), 5.85 (s, 1H), 5.55 (s, 1H), 2.08 (s, 3H).

2-Methyl-*N*-(3,4-di-chloro-phenyl)-acrylamide 5f. Compound **5f** was prepared from coupling 3,4-dichloro-trifluoromethyl-aniline and methyl acrylic acid according to the procedure used to prepare **5b**. ^{1}H NMR ($CDCl_3$) δ 7.85 (s, 1H), 7.50 (s, br, 1H), 7.36 (s, 2H), 5.78 (s, 1H), 5.51 (s, 1H), 2.08 (s, 3H). MS (*m*/*z*): 230, $(M + H)^+$.

2-Methyl-*N*-(4-cyano-phenyl)-acrylamide 5g. Compound **5g** was prepared from coupling 4-cyano-aniline and methyl acrylic acid according to the procedure used to prepare **5b**. Yield, 85%. ^{1}H NMR ($CDCl_3$) δ 7.70 (m, 5H), 5.80 (s, 1H), 5.05 (s, 1H), 2.00 (s, 3H). MS (*m*/*z*): 187, $(M + H)^+$; 209, $(M + Na)^+$.

***N*-(4-Cyano-3-trifluoromethyl-phenyl)-2-ethyl-acrylamide 5i.** Compound **5i** was prepared from coupling 4-cyano-3-trifluoromethyl-aniline and ethyl acrylic acid according to the procedure used to prepare **5b**. Yield, 67%. ^{1}H NMR ($CDCl_3$) δ 8.70 (s, 1H), 8.18 (s, 1H), 8.06 (d, J = 8.0 Hz, 1H), 7.78 (d, J = 8.0 Hz, 1H), 5.75 (s, 1H), 5.05 (s, 1H), 2.40 (q, J = 9.0 Hz, 2H), 1.11 (t, J = 9.0 Hz, 3H). MS (*m*/*z*): 270, $(M + H)^+$; 292, $(M + Na)^+$.

***N*-(4-Cyano-3-trifluoromethyl-phenyl)-2-propyl-acrylamide 5j.** Compound **5j** was prepared from coupling 4-cyano-3-trifluoromethyl-aniline and *n*-propyl acrylic acid according to the procedure used to prepare **5b**. Yield, 58%. ^{1}H NMR ($CDCl_3$) δ 8.20 (s, 1H), 8.12 (s, 1H), 8.00 (d, J = 8.5 Hz, 1H), 7.78 (d, J = 8.5 Hz, 1H), 5.70 (s, 1H), 5.00 (s, 1H), 2.40 (t, J = 9.0 Hz, 2H), 1.50 (m, 2H), 0.95 (t, J = 9.0 Hz, 3H). MS (*m*/*z*): 284, $(M + H)^+$; 306, $(M + Na)^+$.

***N*-(4-Cyano-2-ethyl-3-trifluoromethyl-phenyl)-2-methyl-acrylamide 5k.** Compound **5k** was prepared from coupling 4-cyano-3-trifluoromethyl-2-ethyl-aniline and methyl acrylic acid according to the procedure used to prepare **5b**. Yield, 77%. ^{1}H NMR ($CDCl_3$) δ 8.60 (d, J = 8.5 Hz, 1H), 7.80 (s, 1H), 7.72 (d, J = 8.5 Hz, 1H), 5.88 (s, 1H), 5.63 (s, 1H), 2.85 (q, J = 9.0 Hz, 2H), 2.12 (s, 3H), 1.40 (t, J = 9.0 Hz, 3H). MS (*m*/*z*): 283, $(M + H)^+$.

***N*-(4-Cyano-2-ethyl-5-trifluoromethyl-phenyl)-2-methyl-acrylamide 5l.** Compound **5l** was prepared from coupling 4-cyano-5-trifluoromethyl-2-ethyl-aniline and methyl acrylic acid according to the procedure used to prepare **5b**. Yield, 82%. ^{1}H NMR ($CDCl_3$) δ 8.80 (s, 1H), 7.70 (br, 1H), 7.65 (s, 1H), 5.90 (s, 1H), 5.60 (s, 1H), 3.70 (m, 2H), 2.10 (s, 3H), 1.30 (m, 3H). MS (*m*/*z*): 283, $(M + H)^+$.

***N*-(4-Cyano-2-methoxy-5-trifluoromethyl-phenyl)-2-methyl-acrylamide 5m.** Compound **5m** was prepared from coupling 4-cyano-5-trifluoromethyl-2-methoxy-aniline and methyl acrylic acid according to the procedure used to prepare **5b**. Yield, 75%. ^{1}H NMR ($CDCl_3$) δ 9.00 (s, 1H), 8.40 (s, 1H), 7.26 (s, 1H), 5.90 (s, 1H), 5.60 (s, 1H), 4.00 (s, 3H), 2.10 (s, 3H). MS (*m*/*z*): 285, $(M + H)^+$.

***N*-(2-Chloro-4-cyano-5-trifluoromethyl-phenyl)-2-methyl-acrylamide 5n.** Compound **5n** was prepared from coupling 4-cyano-5-trifluoromethyl-2-chloro-aniline and methyl acrylic acid according to the procedure used to prepare **5b**. Yield, 71%. ^{1}H NMR ($CDCl_3$)

δ 7.95 (s, 1H), 7.52 (s, 1H), 5.80 (br, s, 1H), 5.65 (s, 1H), 5.60 (s, 1H), 2.08 (s, 3H). MS (*m*/*z*): 289, $(M + H)^+$.

***N*-(4-Cyano-2-iodo-5-trifluoromethyl-phenyl)-2-methyl-acrylamide 5o.** Compound **5o** was prepared from coupling 4-cyano-5-trifluoromethyl-2-iodo-aniline and methyl acrylic acid according to the procedure used to prepare **5b**. Yield, 68%. ^{1}H NMR ($CDCl_3$) δ 9.00 (s, 1H), 8.30 (br, 1H), 8.20 (s, 1H), 6.00 (s, 1H), 5.65 (s, 1H), 2.15 (s, 3H). MS (*m*/*z*): 289, $(M - H)^+$.

***N*-(2,4-Dicyano-5-trifluoromethyl-phenyl)-2-methyl-acrylamide 5p.** Compound **5p** was prepared from coupling 2,4-dicyano-5-trifluoromethyl-aniline and methyl acrylic acid according to the procedure used to prepare **5b**. Yield, 65%. ^{1}H NMR ($CDCl_3$) δ 9.15 (s, 1H), 8.45 (br, s, 1H), 8.08 (s, 1H), 6.05 (s, 1H), 5.75 (s, 1H), 2.12 (s, 3H). MS (*m*/*z*): 280, $(M + H)^+$.

***N*-(4-Cyano-2-ethylsulfanyl-5-trifluoromethyl-phenyl)-2-methyl-acrylamide 5q.** Compound **5q** was prepared from coupling 4-dicyano-5-trifluoromethyl-2-ethylsulfanyl-aniline and methyl acrylic acid according to the procedure used to prepare **5b**. Yield, 56%. ^{1}H NMR ($CDCl_3$) δ 9.10 (br, s, 1H), 9.05 (s, 1H), 7.88 (s, 1H), 5.98 (s, 1H), 5.60 (s, 1H), 2.95 (q, $J = 9.5$ Hz, 2H), 2.12 (s, 3H), 1.32 (t, $J = 9.5$ Hz, 3H). MS (*m*/*z*): 315, $(M + H)^+$.

***N*-(4-Cyano-3-trifluoromethyl-phenyl)-2-trifluoromethyl-acrylamide 5h.** The title compound was prepared using different procedure as above. 2-Trifluoromethyl-acrylic acid (5.04 g, 36.0 mmol) in thionyl chloride (2.86 mL) was refluxed for 30 min. Extra thionyl chloride was removed in vacuo. 4-Amino-2-trifluoromethyl-benzonitrile (6.70 g, 36.0 mmol) in ether (50 mL) was added dropwise into the above residue at −40 °C. The reaction was slowly warm to room temperature. The reaction mixture was partitioned between Et_2O and water. The Et_2O layer was washed with sat. sodium bicarbonate and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated to yield a brown oil. The crude material (the brown oil) was then purified by CombiFlash system (silica gel, EtOAc as eluent) to yield the title compound as yellow solid along with a hydration side product as *N*-(4-cyano-3-trifluoromethyl-phenyl)-3,3,3-trifluoro-2-hydroxymethyl-propionamide (~3.2 g). Yield was not calculated since the side product contaminated the desired product, however, it would not affect the next step reaction. ^{1}H NMR ($CDCl_3$) δ 8.25 (br, s, 1H), 7.60 (d, $J = 8.0$ Hz,1H), 6.95 (s, 1H), 6.75 (d, $J = 8.0$ Hz, 1H), 6.25 (s, 1H), 5.98 (s, 1H).

Typical Procedure for Preparation of Pyrazolines 6. Toluenesulfonylhydrazones were prepared by coupling of corresponding aldehydes and hydrazines in MeOH.

5-(4-Fluoro-phenyl)-3-methyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide 6h. 2-[(1*E*)-(4-Fluorophenyl)methylidene]toluenesulfonylhydrazone (600 mg, 2.1 mmol) in THF (20 mL) was treated by NaH (60%, 120 mg, 3 mmol) at 0 °C for 20 min, followed by the addition of *N*-(4-cyano-3-trifluoromethyl-phenyl)-2-methyl-acrylamide (500 mg, 2.0 mmol). The reaction mixture was then heated to 55 °C overnight, quenched by $NaHCO_3$, and extracted by ethyl acetate. The organic layer was combined, washed with brine, dried over Na_2SO_4, and concentrated to yield crude product. Purification of the crude product on CombiFlash system (silica gel, from pure hexanes to 1:1 hexanes:ethyl acetate) yielded the title compound as a white solid and its regioisomer **11** (5:1 ratio). Compound **11** was then stirred in trifluoroacetic acid at room temperature for 30 min. The solvent was removed and the resulting foam was recrystallized from hexanes and ethyl acetate to afford the title compound (580 mg). Total yield, 71%. ^{1}H NMR ($CDCl_3$) δ 9.75 (s, 1H), 8.10 (s, 1H), 7.85 (dd, $J = 7.5$ Hz, 2.0 Hz, 2H), 7.65 (m, 2H), 7.05 (m, 2H), 5.70 (br, 1H), 3.30 (abq, $J = 10.6$ Hz, 2 H), 1.65 (s, 3H). MS (*m*/*z*): 391, $(M + H)^+$. Anal. ($C_{19}H_{14}F_4N_4O$.0.7MeOH) C, H, N.

5-(4-Acetylamino-phenyl)-2,3-dimethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Nitro-3-trifluoromethyl-phenyl)-amide 6a. Compound **6a** was prepared from coupling 2-[(1*E*)-(4-acetamidophenyl)methylidene]toluenesulfonyl-hydrazone and **5a** according to the procedure used to prepare **6h**. Yield, 65%. ^{1}H NMR ($CDCl_3$) δ 9.52 (s, 1H), 8.15 (s, 1H), 8.06 (d, $J = 7.5$ Hz, 1H), 7.95 (d, $J = 7.5$ Hz, 1H), 7.61 (s, 1H), 7.55 (m, 4H), 3.38 (abq, $J = 12.5$ Hz, 2H), 2.18 (s, 3H), 1.48 (s, 3H). MS (*m*/*z*): 464, $(M + H)^+$, 486 $(M + Na)^+$. HRMS calculated for $C_{20}H_{18}F_3N_5O_4$ $(M + H)^+$: 430.1491; found: 430.1489.

5-(4-Acetylamino-phenyl)-2,3-dimethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide 6b. Compound **6b** was prepared from coupling 2-[(1*E*)-(4-acetamidophenyl)methylidene]toluenesulfonyl-hydrazone and **5b** according to the procedure used to prepare **6h**. Yield, 70%. ^{1}H NMR ($CDCl_3$) δ 9.62 (s, 1H), 8.18 (s, 1H), 8.02 (d, $J = 7.5$ Hz, 1H), 7.98 (d, $J = 7.5$ Hz, 1H), 7.80 (s, 1H), 7.50 (m, 4H), 3.38 (abq, $J = 12.5$ Hz, 2H), 2.20 (s, 3H), 1.50 (s, 3H). MS (*m*/*z*): 444, $(M + H)^+$. Anal. ($C_{21}H_{18}F_3N_5O_2$) C, H, N.

3-Methyl-5-[4-(2,2,2-trifluoro-acetylamino)-phenyl]-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Nitro-3-trifluoromethyl-phenyl)-amide 6c. Compound **6c** was prepared from coupling 2-[(1*E*)-(4-trifluoromethylacetamidophenyl)methylidene]-toluenesulfonyl-hydrazone and **5a** according to the procedure used to prepare **6h**. Yield, 52%. ^{1}H NMR (C_6D_6) δ 8.95 (s, 1H), 7.62 (s, 1H), 7.50 (d, $J = 7.8$ Hz, 2H), 7.35 (d, $J = 7.0$ Hz, 1H), 7.20 (d, $J = 7.5$ Hz, 2H), 7.18 (d, $J = 7.0$ Hz, 1H), 4.95 (s, 1H), 2.80 (abq, $J = 15.6$ Hz, 2H), 1.62 (s, 3H). MS (*m*/*z*): 504, $(M + H)^+$. HRMS calculated for $C_{20}H_{15}F_6N_5O_4$ $(M + H)^+$: 504.1106; found: 504.1091.

5-(4-Acetylamino-phenyl)-3-methyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (3,4-Dicyano-phenyl)-amide 6d. Compound **6d** was prepared from coupling 2-[(1*E*)-(4-acetamidophenyl)methylidene]toluenesulfonyl-hydrazone and **5c** according to the procedure used to prepare **6h**. Yield, 68%. ^{1}H NMR ($CDCl_3$) δ 9.79 (s, br, 1H), 8.25 (s, 1H), 7.88 (d, $J = 6.8$ Hz, 1H), 7.72 (d, $J = 6.8$ Hz, 1H), 7.55 (s, 4H), 5.68 (s, 1H), 3.35 (abq, $J = 12.5$ z, 2H), 2.28 (s, 3H), 1.68 (s, 3H). MS (*m*/*z*): 387, $(M + H)^+$. HRMS calculated for $C_{21}H_{18}F_6N_6O_2$ $(M + H)^+$: 387.1569; found: 387.1566.

5-(4-Acetylamino-phenyl)-3-methyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Chloro-3-trifluoromethyl-phenyl)-amide 6e. Compound **6e** was prepared from coupling 2-[(1*E*)-(4-acetamidophenyl)methylidene]toluenesulfonyl-hydrazone and **5d** according to the procedure used to prepare **6h**. Yield, 50%. ^{1}H NMR ($CDCl_3$) δ 9.50 (s, 1H), 8.00 (s, 1H), 7.90 (s, 1H), 7.75 (m, 1H), 7.50 (s, 4H), 7.45 (m, 1H), 5.70 (s, 1H), 3.25 (abq, $J = 12.5$ Hz, 2H), 2.15 (s, 3H), 1.60 (s, 3H). MS (*m*/*z*): 439, $(M + H)^+$. Anal. ($C_{20}H_{18}ClF_3N_4O_2$) C, H, N.

5-(3-Acetylamino-phenyl)-3-methyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide 6f. Compound **6f** was prepared from coupling 2-[(1*E*)-(3-acetamidophenyl)methylidene]toluenesulfonyl-hydrazone and **5a** according to the procedure used to prepare **6h**. Yield, 52%. ^{1}H NMR ($CDCl_3$) δ 9.75 (s, 1H), 8.15 (s, 1H), 8.00−7.75 (m, 4H), 7.40 (m, 1H), 7.25 (s, 1H), 5.80 (s, 1H), 3.25 (abq, $J = 15.4$, 2H), 2.20 (s, 3H), 1.60 (s, 3H). MS (*m*/*z*): 431, $(M + H)^+$. Anal. ($C_{21}H_{18}F_3N_5O_2$) C, H, N.

5-(4-Acetylamino-benzyl)-3-methyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide 6g. Compound **6g** was prepared from coupling 2-[(1*E*)-(4-acetamidobenzyl)methylidene]toluenesulfonyl-hydrazone and **5b** according to the procedure used to prepare **6h**. Yield, 48%.^{1}H NMR ($CDCl_3$) δ 9.80 (s, 1H), 8.10 (s, 1H), 8.00 (s, 1H), 7.95 (m, 1H), 7.75 (s, 1H), 7.55 (s, 4H), 5.75 (s, 1H), 3.30 (abq, $J = 12.4$ Hz, 2H), 2.20 (s, 2H), 1.60 (s, 3H). MS (*m*/*z*): 468, $(M + Na)^+$. Anal. ($C_{22}H_{20}F_3N_5O_2$. 0.35EtOAc) C, H, N.

5-(4-Fluoro-phenyl)-3-methyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Nitro-3-trifluoromethyl-phenyl)-amide 6i. Compound **6i** was prepared from coupling 2-[(1*E*)-(4-fluorophenyl)-methylidene]toluenesulfonyl-hydrazone and **5a** according to the procedure used to prepare **6h.** Yield, 56%. ^{1}H NMR (MeOH) δ 6.45 (d, $J = 6.9$ Hz, 1H), 6.20 (m, 1H), 6.00 (s, 1H), 5.55 (m, 4H), 2.00 (abq, $J = 11.8$ Hz, 2 H), 1.70 (s, 3H). MS (*m*/*z*): 410, $(M + Na)^+$. Anal. ($C_{18}H_{14}F_4N_4O_3$·$0.2H_2O$) C, H, N.

3-Methyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide 7a. *N*-(4-Cyano-3-trifluoromethyl-phenyl)-2-methyl-acrylamide **5b** (180 mg, 0.71 mmol) in THF (5 mL) was treated with $TMSCHN_2$ (2.0 M in hexanes, 3.54

mmol, 1.8 mL) at −10 °C. The reaction mixture was then warmed to room-temperature slowly and stirred overnight. The solvent was removed and the residue was stirred in trifluoroacetic acid (2 mL) at room temperature over 2 h. The solvent was removed and the residue purified by silica gel cartridge on CombiFlash system using eluent gradient from hexanes:ethyl acetate 5:1 to 1:1 to yield the title compound as a white solid (164 mg). Yield, 78%. ^{1}H NMR ($CDCl_3$) δ 9.62 (s, 1H), 8.10 (s, 1H), 7.95 (d, J = 6.5 Hz, 1H), 7.77 (d, J = 6.5 Hz, 1H), 6.88 (s, 1H), 5.52 (s, 1H), 3.05 (abq, J = 12.5 Hz, 2H), 1.56 (s, 3H). MS (*m*/*z*): 297, $(M + H)^+$. HRMS calculated for $C_{13}H_{11}F_3N_4O$ $(M + H)^+$: 297.0963; found: 297.0978.

3,5-Dimethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide 7b. Diazoethane (∼ 0.5 M, 20 mL) in diethyl ether was added into 2-methyl-*N*-(4-cyano-3-trifluoromethyl-phenyl)-acrylamide **5b** (250 mg, 1 mmol) in THF (2 mL) at room temperature. The solution was stirred at room temperature for 72 h. The solvent was removed, and the residue was stirred in trifluoroacetic acid (5 mL) at room temperature over 2 h. The solvent was removed and the residue purified by silica gel cartridge on CombiFlash system using eluent gradient from hexanes:ethyl acetate 5:1 to 1:1 to yield the title compound as a white solid (158 mg). Yield, 51%. ^{1}H NMR ($CDCl_3$) δ 9.85 (s, br, 1H), 8.05 (s, 1H), 7.95 (d, J = 7.5 Hz, 1H), 7.75 (d, J = 7.5 Hz, 1H), 2.95 (abq, J = 12.5 Hz, 2H), 1.98 (s, 3H), 1.55 (s, 3H). MS (*m*/*z*): 311 $(M + H)^+$. Anal. ($C_{14}H_{13}F_3N_4O$) C, H, N.

3-Methyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide 7c. 2-[(1*E*)-Trifluoromethyl-methylidene]toluenesulfonyl-hydrazone (780 mg, 2.93 mmol) in THF (20 mL) was treated by NaH (60%, 176 mg, 4.40 mmol) at 0 °C for 20 min, followed by the addition of *N*-(4-cyano-3-trifluoromethyl-phenyl)-2-methyl-acrylamide **5b** (744 mg, 2.93 mmol). The reaction mixture was then heated to 55 °C overnight, quenched by $NaHCO_3$, and extracted by ethyl acetate. The organic layer was combined, washed with brine, dried over Na_2SO_4, and concentrated to yield crude product. Purification of the crude product on CombiFlash system (silica gel, from pure hexanes to pure ethyl acetate) yielded the title compound as a white solid. Depending on the substrates, sometimes it is the regioisomer **11** could be detected from the reaction along with the desired product. Thus, **11** was then stirred in trifluoroacetic acid at room temperature for 30 min. The solvent was removed, and the resulting solid was recrystallized from hexanes and ethyl acetate to afford the title compound (864 mg). Yield, 81%. ^{1}H NMR ($CDCl_3$) δ 9.30 (s, 1H), 8.11 (s, 1H), 7.98 (dd, J = 7.1 Hz, 1.2 Hz, 1H), 7.80 (d, J = 7.8 Hz, 1H), 6.18 (br, 1H), 3.15 (abq, J = 11.0 Hz, 2 H), 1.62 (s, 3H). MS (*m*/*z*): 387, $(M + Na)^+$. Anal. ($C_{14}H_{10}F_6N_4O$) C, H, N.

(*R*)-3-Methyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide (*R*)-7c and (*S*)-3-Methyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide (*S*)-7c. A racemic mixture of 3-methyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic acid (4-cyano-3-trifluoromethyl-phenyl)-amide **7c** (500 mg) was loaded onto a ChiralPak AD chiral HPLC column (50 mm I.D. × 500 mm L) and eluted with 10% ethanol in heptane at the 70 mL/min flow rate. Two peaks were collected separately and were removed under vacuum to yield: (*S*)-**7c** as peak one $[\alpha]_D$ −4.2° (MeOH); MS (*m*/*z*): 365, $(M + H)^+$] (∼ 225 mg, 45% recovery) and (*R*)-**7c** as peak two [$[\alpha]_D$ +5.7° (MeOH);MS (*m*/*z*): 365, $(M + H)^+$] (∼ 170 mg, 35% recovery).

3-Methyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Nitro-3-trifluoromethyl-phenyl)-amide 7d. Compound **7d** was prepared from coupling 2-[(1*E*)-(trifluoromethyl)-methylidene]toluenesulfonyl-hydrazone and **5a** according to the procedure used to prepare **7c**. Yield, 55%. ^{1}H NMR ($CDCl_3$) δ 9.30 (s, 1H), 8.10 (s, 1H), 8.00 (m, 2H), 6.10 (s, 1H), 3.15 (abq, J = 12.4 Hz, 2H), 1.66 (s, 3H). MS (*m*/*z*): 385, $(M + H)^+$. Anal. ($C_{13}H_{10}F_6N_4O$•$1.2H_2O$) C, H, N.

3-Methyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Chloro-3-trifluoromethyl-phenyl)-amide 7e. Compound **7e** was prepared from coupling 2-[(1*E*)-(trifluoromethyl)methylidene]toluenesulfonyl-hydrazone and **5d** according to the procedure used to prepare **7c**. Yield, 68%. ^{1}H NMR ($CDCl_3$) δ 8.95 (s, 1H), 7.95 (s, 1H), 7.75 (m, 1H), 7.50 (m, 1H), 6.00 (s, 1H), 3.15 (abq, J = 10.5 Hz, 2H), 1.60 (s, 3H). MS (*m*/*z*): 374, $(M + H)^+$. Anal. ($C_{13}H_{10}ClF_6N_4O_3$) C, H, N.

3-Methyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Bromo-3-trifluoromethyl-phenyl)-amide 7f. Compound **7f** was prepared from coupling 2-[(1*E*)-(trifluoromethyl)methylidene]toluenesulfonyl-hydrazone and **5e** according to the procedure used to prepare **7c**. Yield, 66%. ^{1}H NMR ($CDCl_3$) δ 8.95 (s, 1H), 7.96 (s, 1H), 7.65 (s, 2H), 5.86 (s, 1H), 3.19 (abq, J = 9.8 Hz, 2H), 1.60 (s, 3H). MS (*m*/*z*): 419 $(M + H)^+$. HRMS calculated for $C_{13}H_{10}BrF_6N_3O$ $(M + H)^+$: 417.9989; found: 418.0005.

3-Methyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (3,4-Dichloro-phenyl)-amide 7g. Compound **7g** was prepared from coupling 2-[(1*E*)-(trifluoromethyl)methylidene]-toluenesulfonyl-hydrazone and **5f** according to the procedure used to prepare **7c**. Yield, 61%. ^{1}H NMR ($CDCl_3$) δ 8.85 (s, 1H), 7.85 (s, 1H), 7.40 (m, 2H), 5.85 (s, 1H), 3.15 (abq, J = 10.5 Hz, 2H), 1.60 (s, 3H). MS (*m*/*z*): 341, $(M + H)^+$. Anal. ($C_{12}H_{10}Cl_2F_3N_3O$• 0.2MeOH) C, H, N.

3-Methyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-phenyl)-amide 7h. Compound **7h** was prepared from coupling 2-[(1*E*)-(trifluoromethyl)methylidene]-toluenesulfonyl-hydrazone and **5g** according to the procedure used to prepare **7c**. Yield, 71%. ^{1}H NMR ($CDCl_3$) δ 9.05 (s, 1H), 7.70−7.60 (m, 4H), 5.95 (s, 1H), 3.15 (abq, J = 9.8 Hz, 2H), 1.60 (s, 3H). MS (*m*/*z*): 297, $(M + H)^+$. Anal. ($C_{13}H_{11}F_3N_4O$) C, H, N.

5-Ethyl-3-methyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide 7i. Compound **7i** was prepared from coupling 2-[(1*E*)-ethylmethylidene]toluenesulfonyl-hydrazone and **5b** according to the procedure used to prepare **7c**. Yield, 50%. ^{1}H NMR ($CDCl_3$) δ 9.89 (s, 1H), 8.14 (s, 1H), 7.95 (d, J = 7.6 Hz, 1H), 7.80 (d, J = 7.6 Hz, 1H), 5.33 (m, 1H), 2.95 (abq, J = 10.6 Hz, 2H), 2.35 (m, J = 6.6 Hz, 2H), 1.58 (s, 3H), 1.18 (t, J = 6.6 Hz, 3H). MS (*m*/*z*): 347, $(M + Na)^+$. Anal. ($C_{15}H_{15}F_3N_4O$) C, H, N.

5-Isopropyl-3-methyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide 7j. Compound **7j** was prepared from coupling 2-[(1*E*)-isopropylmethylidene]-toluenesulfonyl-hydrazone and **5b** according to the procedure used to prepare **7c**. Yield, 54%. ^{1}H NMR ($CDCl_3$) δ 9.85 (s, 1H), 8.10 (s, 1H), 7.95 (dd, J = 7.0 Hz, 2.2 Hz, 1H), 7.78 (d, J = 7.8 Hz, 1H), 5.35 (br, 1H), 2.98 (abq, J = 10.4 Hz, 2 H), 2.55 (m, 1H), 1.55 (s, 3H), 1.16 (d, J = 8.5 Hz, 6H). MS (*m*/*z*): 339, $(M + H)^+$. Anal. ($C_{16}H_{17}F_3N_4O$•$0.5H_2O$) C, H, N.

3-Methyl-5-(2,2,2-trifluoro-ethyl)-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide 7k. Compound **7k** was prepared from coupling 2-[(1*E*)-2,2,2-trifluoroethylmethylidene]toluenesulfonyl-hydrazone and **5b** according to the procedure used to prepare **7c**. Yield, 45%. ^{1}H NMR ($CDCl_3$) δ 9.60 (s, 1H), 8.15 (s, 1H), 7.95 (m, 1H), 7.80 (m, 1H), 5.65 (s, 1H), 3.20 (m, 2H), 3.05 (abq, J = 12.4 Hz, 2H), 1.55 (s, 3H). MS (*m*/*z*): 379, $(M + H)^+$. Anal. ($C_{15}H_{12}F_6N_4O$) C, H, N.

3-Methyl-5-pentafluoroethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide 7l. Compound **7l** was prepared from coupling 2-[(1*E*)-pentafluoroethylmethylidene]toluenesulfonyl-hydrazone and **5h** according to the procedure used to prepare **7c**. Yield, 36%. ^{1}H NMR (MeOD) δ 8.21 (s, 1H), 8.10 (d, J = 6.5 Hz, 1H), 7.88 (d, J = 6.5 Hz, 1H), 3.30 (abq, J = 12.5 Hz, 2H), 1.68 (s, 3H). MS (*m*/*z*): 415, $(M + H)^+$. Anal. ($C_{15}H_{12}F_6N_4O$) C, H, N.

3,5-Bis-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide 7m. CF_3-CHN_2 was prepared by the literature known method and stored under 0 °C in anhydrous ether solution (∼ 0.5 M). More than 10.0 equiv of CF_3CHN_2 ether solution was added into **5h** (250 mg, 0.81 mmol) in THF (10 mL) at 0 °C. The reaction was then slowly warm to room temperature and stirred for another 4 h. After solvent was removed, trifluoroacetic acid (2 mL) was added to the residue and

stirred for another 30 min. The solvent was removed and the crude product was purified by CombiFlash system using silica gel cartridge with hexanes and ethyl acetate as eluent to give the title compound as a white solid (∼ 108 mg). Yield, ∼ 32%. ^{1}H NMR ($CDCl_3$) δ 9.18 (s, 1H), 8.11 (s, 1H), 8.05 (d, J = 8.0 Hz, 1H), 7.82 (d, J = 8.0 Hz, 1H), 7.05 (s, 1H), 3.35 (abq, J = 9.0 Hz, 2H). MS (*m*/*z*): 419 $(M + H)^+$. Anal. ($C_{14}H_7F_9N_4O$) C, H, N.

5-Methyl-3-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide 7n. Compound **7n** was prepared from coupling 2-[(1*E*)-trifluoromethylmethylidene]toluenesulfonyl-hydrazone and **5h** according to the procedure used to prepare **7b**.^{1}H NMR ($CDCl_3$) δ 9.75 (br, s, 1H), 8.15 (s, 1H), 7.98 (d, J = 6.5 Hz, 1H), 7.80 (d, J = 7.5 Hz, 1H), 6.25 (s, 1H), 3.45 (abq, J = 8.5 Hz, 1H), 3.25 (abq, J = 8.5 Hz, 1H), 2.12 (s, 3H). MS (*m*/*z*): 365, $(M + H)^+$. Anal. ($C_{14}H_{10}F_6N_4O$) C, H, N.

3-Ethyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide 7p. Compound **7p** was prepared from coupling 2-[(1*E*)-trifluoromethylmethylidene]toluenesulfonyl-hydrazone and **5i** according to the procedure used to prepare **7c**. ^{1}H NMR ($CDCl_3$) δ 9.37 (s, 1H), 8.11 (s, 1H), 7.95−7.80 (m, 2H), 6.10 (s, 1H), 3.22 (dd, J = 6.0, 2.7 Hz, 2 H), 2.05 (m, 2H), 1.00 (t, J = 7.5 Hz, 3H). MS (*m*/*z*): 379, $(M + H)^+$. HRMS calculated for $C_{15}H_{14}F_6N_4O$ $(M + H)^+$: 379.0994; found: 379.1000.

3-Propyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide 7q. Compound **7q** was prepared from coupling 2-[(1*E*)-trifluoromethylmethylidene]toluenesulfonyl-hydrazone and **5j** according to the procedure used to prepare **7c**. ^{1}H NMR ($CDCl_3$) δ 9.30 (s, 1H), 8.15 (s, 1H), 7.95 (m, 1H), 7.80 (m, 1H), 6.25 (s, 1H), 3.15 (dd, J = 8.0, 2.7 Hz, 2H), 2.00 (m, 2H), 1.30 (m, 2H), 1.65 (t, J = 8.0 Hz, 3H). MS (*m*/*z*): 393, $(M + H)^+$. Anal. ($C_{16}H_{14}F_6N_4O$) C, H, N.

3-Methyl-5-*N*-propyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide 7r. Compound **7r** was prepared from coupling 2-[(1*E*)-*n*-propylmethylidene]-toluenesulfonyl-hydrazone and **5b** according to the procedure used to prepare **7c**. Yield, 45%. ^{1}H NMR ($CDCl_3$) δ 9.88 (s, 1H), 8.13 (s, 1H), 7.95 (d, J = 7.6 Hz, 1H), 7.79 (d, J = 7.6 Hz, 1H), 5.39 (s, 1H), 2.90 (abq, J = 12.0 Hz, 2H), 2.29 (t, J = 6.6 Hz, 2H), 1.57 (m, 2H), 1.56 (s, 3H), 0.95 (t, J = 6.5 Hz, 3H). MS (*m*/*z*): 339, $(M + H)^+$. Anal. ($C_{16}H_{17}F_3N_4O{\cdot}0.5C_5H_5N$) C, H, N.

5-*tert*-Butyl-3-methyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide 7s. Compound **7s** was prepared from coupling 2-[(1*E*)-*tert*-butylmethylidene]-toluenesulfonyl-hydrazone and **5b** according to the procedure used to prepare **7c.** Yield, 70%. ^{1}H NMR ($CDCl_3$) δ 9.78 (br, s, 1H), 8.12 (s, 1H), 7.92 (d, J = 7.5 Hz, 1H), 7.78 (d, J = 7.5 Hz, 1H), 5.25 (br, s, 1H), 2.95 (abq, J = 12.5 Hz, 2H), 1.58 (s, 3H), 1.15 (s, 9H). MS (*m*/*z*): 353, $(M + H)^+$. Anal. ($C_{17}H_{19}F_3N_4O$) C, H, N.

5-(4-Cyano-3-trifluoromethyl-phenylcarbamoyl)-5-methyl-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Ethyl Ester 7o. N_2-$CHCO_2Et$ (223 mg, 1.96 mmol) was added into **5b** (250 mg, 0.98 mmol) in THF (10 mL) at 0 °C. The reaction was then slowly warm to room temperature and stirred for another 4 h. After solvent was removed, trifluoroacetic acid (2 mL) was added to the residue and stirred for another 30 min. The solvent was removed, and the crude product was purified by CombiFlash system using silica gel cartridge with hexanes and ethyl acetate as eluent to give the title compound as a white solid (240 mg). Yield, 67%. ^{1}H NMR ($CDCl_3$) δ 99.18 (s, br, 1H), 8.11 (s, 1H), 7.98 (d, J = 7.2 Hz, 1H), 7.81 (d, J = 7.2 Hz, 1H), 6.25 (s, 1H), 4.32 (q, J = 8.5 Hz, 2H), 3.25 (abq, J = 12.5 Hz, 2H), 1.62 (s, 3H), 1.45 (t, J = 8.5 Hz, 3H). MS (*m*/*z*): 369 $(M + H)^+$. Anal. ($C_{16}H_{15}F_3N_4O_3{\cdot}0.25H_2O$) C, H, N.

3-Methyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-2-ethyl-3-trifluoromethyl-phenyl)-amide 8a. Compound **8a** was prepared from coupling 2-[(1*E*)-trifluoromethylmethylidene]toluenesulfonyl-hydrazone and **5k** according to the procedure used to prepare **7c**. Yield, 65%. ^{1}H NMR (MeOD) δ 9.50 (s, 1H), 8.60 (d, J = 7.8 Hz, 1H), 7.70 (d, J = 1.8 Hz, 1H), 5.90 (s, 1H), 3.30 and 3.05 (abq, J = 12.0 Hz, 2H), 2.80 (m, 2H), 1.65 (s, 3H), 1.20 (m, 3H). MS (*m*/*z*): 393, $(M + H)^+$. Anal. ($C_{14}H_{12}F_3N_3O_2$) C, H, N.

3-Methyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-2-ethyl-5-trifluoromethyl-phenyl)-amide 8b. Compound **8b** was prepared from coupling 2-[(1*E*)-trifluoromethylmethylidene]toluenesulfonyl-hydrazone and **5l** according to the procedure used to prepare **7c**. Yield, 73%. ^{1}H NMR ($CDCl_3$) δ 9.30 (s, 1H), 8.80 (s, 1H), 7.55 (s, 1H), 5.90 (s, 1H), 3.25 and 3.10 (abq, J = 14.0 Hz, 2H), 2.70 (m, 2H), 1.65 (s, 3H), 1.30 (m, 3H). MS (*m*/*z*): MH^+ 393, $(M + H)^+$. Anal. ($C_{14}H_{12}F_3N_3O_2$) C, H, N.

3-Methyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-2-methoxy-5-trifluoromethyl-phenyl)-amide 8c. Compound **8c** was prepared from coupling 2-[(1*E*)-trifluoromethylmethylidene]toluenesulfonyl-hydrazone and **5m** according to the procedure used to prepare **7c**. Yield, 68%. ^{1}H NMR ($CDCl_3$) δ 9.65 (br, 1H), 8.90 (s, 1H), 7.25 (s, 1H), 5.90 (s, 1H), 4.00 (s, 3H), 3.25 and 3.05 (abq, J = 10.0 Hz, 2H), 1.60 (s, 3H). MS (*m*/*z*): 395, $(M + H)^+$. Anal. ($C_{15}H_{12}F_6N_4O_2{\cdot}0.2$EtOAc) C, H, N.

3-Methyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (2-chloro-4-cyano-5-trifluoromethyl-phenyl)-amide 8d. Compound **8d** was prepared from coupling 2-[(1*E*)-trifluoromethylmethylidene]toluenesulfonyl-hydrazone and **5n** according to the procedure used to prepare **7c**. Yield 52%. ^{1}H NMR ($CDCl_3$) δ 9.88 (br, s, 1H), 9.05 (s, 1H), 7.90 (s, 1H), 3.31 (abq, J = 10.5 Hz, 1H), 3.15 (abq, J = 11.0 Hz, 1H), 1.68 (s, 3H). MS (*m*/*z*): 399, $(M + H)^+$. HRMS calculated for $C_{14}H_9ClF_6N_4O$ $(M + H)^+$: 399.0447; found: 399.0454.

3-Methyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-2-iodo-5-trifluoromethyl-phenyl)-amide 8e. Compound **8e** was prepared from coupling 2-[(1*E*)-trifluoromethylmethylidene]toluenesulfonyl-hydrazone and **5o** according to the procedure used to prepare **7c**. Yield, 75%. ^{1}H NMR ($CDCl_3$) δ 9.80 (s, 1H), 9.10 (s, 1H), 8.20 (s, 1H), 6.00 (s, 1H), 3.25 and 3.10 (abq, J = 14.5 Hz, 2H), 1.65 (s, 3H). MS (*m*/*z*): 491, $(M + H)^+$. Anal. ($C_{14}H_9IF_6N_4O$) C, H, N.

3-Methyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (2,4-Dicyano-5-trifluoromethyl-phenyl)-amide 8f. Compound **8f** was prepared from coupling 2-[(1*E*)-trifluoromethylmethylidene]toluenesulfonyl-hydrazone and **5p** according to the procedure used to prepare **7c**. Yield 55%. ^{1}H NMR ($CDCl_3$) δ 10.10 (s, 1H), 9.10 (s, 1H), 8.10 (s, 1H), 6.45 (s, 1H), 3.30 and 3.10 (abq, J = 14.0 Hz, 2H), 1.65 (s, 3H). MS (*m*/*z*): 390, $(M + H)^+$. Anal. ($C_{15}H_9F_6N_5O$) C, H, N.

3-Methyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-2-ethylsulfanyl-5-trifluoromethyl-phenyl)-amide 8g. Compound **8g** was prepared from coupling 2-[(1*E*)-trifluoromethylmethylidene]toluenesulfonyl-hydrazone and **5q** according to the procedure used to prepare **7c**. Yield, 67%. ^{1}H NMR ($CDCl_3$) δ 8.30 (br, 1H), 7.35 (m, 1H), 7.10 (s, 1H), 6.80 (m, 1H), 5.10 (br, 1H), 3.25 and 3.10 (abq, J = 11.0 Hz, 2H), 1.60 (s, 3H). MS (*m*/*z*): 365, $(M + H)^+$. HRMS calculated for $C_{16}H_{14}F_6N_4OS$ $(M + H)^+$: 425.0870; found: 425.0875.

2,3-Dimethyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide 8a. 3-Methyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic acid (4-cyano-3-trifluoromethyl-phenyl)-amide **7c** (635 mg, 2.5 mmol) in DCM (25 mL) at 0 °C was treated with diethylpropylamine (1.75 mL, 10 mmol) followed by methyl triflate (283 μL, 2.5 mmol). The reaction was gradually warmed to room temperature and stirred overnight. The reaction mixture was partitioned between DCM and water. The DCM layer was washed with sat. sodium bicarbonate and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated to yield a yellow oil, which was then purified by CombiFlash system (silica gel, EtOAc as eluent) to yield the title compound as off-white solid (425 mg). Yield, 45%. ^{1}H NMR ($CDCl_3$) δ 9.02 (s, 1H), 8.11 (s, 1H), 7.98 (d, J = 7.5 Hz, 1H), 7.82 (d, J = 7.5 Hz, 1H), 3.32 (abq, J = 9.5 Hz, 1H), 3.02 (abq, J = 9.5 Hz, 1H), 3.01 (s, 3H), 1.52 (s, 3H). MS (*m*/*z*): 379 $(M + H)^+$. Anal. ($C_{15}H_{12}F_6N_4O$) C, H, N.

2-Ethyl-3-methyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide 9b and *N*-(4-Cyano-3-trifluoromethyl-phenyl)-3-methyl-5-trifluoromethyl-4,5-dihydro-3*H*-pyrazole-3-carboximidic Acid Ethyl Ester 10b. 3-Methyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic acid (4-cyano-3-trifluoromethyl-phenyl)-amide **7c** (400 mg, 1.1 mmol) and Na_2HPO_4 (1.0 g, 7 mmol) in CH_2Cl_2 (10 mL) was treated with Et_3O^+ BF_4^- (1 M in CH_2Cl_2, 5.0 mL) at 0 °C. The reaction mixture was warmed up to r.t. and stirred overnight and then quenched with $NaHCO_3$. CH_2Cl_2 was added to extract the product, and organic layers were washed with brine and dried over Na_2SO_4. Upon the purification by silica gel cartridge on Combiflash system (CH_2Cl_2:EtOAc = 10: 1), the title compounds were obtained as white solids.

9b: Yield, 130 mg, 30%. ^{1}H NMR ($CDCl_3$) δ 9.10 (br, 1H), 8.10 (s, 1H), 7.95 (m, 1H), 7.80 (m, 1H), 3.40−3.00 (m, 4H), 1.50 (s, 3H), 1.35 (m, 3H). MS (*m*/*z*): 393, $(M + H)^+$. Anal. ($C_{16}H_{14}F_6N_4O$) C, H, N.

10b: Yield, 130 mg, 30%. Diastereomers: major peaks in ^{1}H NMR ($CDCl_3$) δ 7.85 (m, 1H), 7.65 (s, 1H), 7.50 (s, 1H), 5.30 (m, 1H), 4.40 (m, 1H), 3.90 (m, 1H), 2.95 (m, 1H), 2.40 (m, 1H), 1.55 (m, 3H), 1.50 (s, 3H). MS (*m*/*z*): 393, $(M + H)^+$. HRMS calculated for $C_{16}H_{14}F_6N_4O$ $(M + H)^+$: 393.1072; found: 393.1078.

(*S*)-2-Ethyl-3-methyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide [(*S*)-9b] and (*S*)-*N*-(4-Cyano-3-trifluoromethyl-phenyl)-3-methyl-5-trifluoromethyl-4,5-dihydro-3*H*-pyrazole-3-carboximidic Acid Ethyl Ester [(*S*)-10b]. Compounds (*S*)-**9b** and (*S*)-**10b** were prepared from (*S*)-**7c** according to the procedure used to prepare **9b** and **10b** [(*S*)-**9b**:(*S*)-**10b** ∼ 1:1, yield 65%]. (*S*)-**9b**: $[\alpha]_D$ −34.2° (MeOH); (*S*)-**10b**: $[\alpha]_D$ +17.6° (MeOH).

(*R*)-2-Ethyl-3-methyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide [(*R*)-9b] and (*R*)-*N*-(4-Cyano-3-trifluoromethyl-phenyl)-3-methyl-5-trifluoromethyl-4,5-dihydro-3*H*-pyrazole-3-carboximidic Acid Ethyl Ester [(*R*)-10b]. Compounds (*R*)-**9b** and (*R*)-**10b** were prepared from (*S*)-**7c** according to the procedure used to prepare **9b** and **10b** [(*R*)-**9b**:(*R*)-**10b** ∼ 1:1, yield 61%]. (*R*)-**9b**: $[\alpha]_D$ +58.2° (MeOH); (*R*)-**10b**: $[\alpha]_D$ +21.5° (MeOH).

5-(4-Acetylamino-phenyl)-2,3-dimethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide 9c. 4-Acetamido-*N*-methyl-benzenecarbohydrazonoyl chloride was prepared by the known literature method. Thus, NCS (1.33 g, 10.0 mmol) was mixed with dimethyl sulfide (620 mg, 10.0 mmol) in CH_2Cl_2 (20 mL) at 0 °C for 30 min. The mixture was then cooled to −78 °C, and *N*-methyl-*N*′-(4-acetamido-benzylidene)-hydrazine (1.91 g, 10.0 mmol) was added into the mixture. The mixture was maintained at −78 °C for 1 h and then slowly warmed up to room temperature over 2 h. The reaction mixture was quenched by $NaHCO_3$ and then extracted with ethyl acetate. The organic layer was combined, washed with brine, dried over Na_2SO_4, and concentrated to yield 4-acetamido-*N*-methyl-benzenecarbohydrazonoyl chloride. *N*-(4-Cyano-3-trifluoromethyl-phenyl)-2-methyl-acrylamide **5b** (890 mg, 3.5 mmol) was mixed with 4-acetamido-*N*-methyl-benzenecarbohydrazonoyl chloride (788 mg, 4.0 mmol) in CH_2Cl_2 at 0 °C. Triethyl amine (∼ 1 mL, 7.0 mmol) was then added to the reaction mixture. The reaction was warmed to room temperature and then refluxed overnight, quenched with $NaHCO_3$, and extracted with ethyl acetate. The organic layer was combined, washed with brine, dried over Na_2SO_4, and concentrated to yield a crude product. Purification of the crude product on CombiFlash system using silica gel cartridge by hexanes and ethyl acetate as eluent yielded the title compound as a white solid (790 mg). Yield, 51%. ^{1}H NMR ($CDCl_3$) δ 9.62 (s, 1H), 8.18 (s, 1H), 8.02 (d, J = 7.5 Hz, 1H), 7.98 (d, J = 7.5 Hz, 1H), 7.80 (s, 1H), 7.50 (m, 4H), 3.38 (abq, J = 12.5 Hz, 2H), 2.98 (s, 3H), 2.20 (s, 3H), 1.50 (s, 3H). MS (*m*/*z*): 444, $(M + H)^+$. Anal. ($C_{22}H_{20}F_3N_5O_2 \cdot 0.5H_2O$) C, H, N.

2-Ethyl-3,5-dimethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide 9d. Compound **9d** was prepared from coupling 4-methyl-*N*-ethyl-carbohydrazonoyl chloride (NCS and Me_2S procedure) and **5b** according to the procedure used to prepare **9c**. Yield 55%. ^{1}H NMR ($CDCl_3$) δ 9.68 (s, 1H), 8.07 (s, 1H), 7.85 (d, J = 8.0 Hz, 1H), 7.75 (d, J = 8.0 Hz, 1H), 3.15 (m, 1H), 3.05 (abq, J = 10.0 Hz, 1H), 2.80 (m, 1H), 2.71 (abq, J = 10.0 Hz, 1H), 1.98 (s, 3H), 1.40 (s, 3H), 1.35 (t, J = 9.5 Hz, 3H). MS (*m*/*z*): 339, $(M + H)^+$. Anal. ($C_{16}H_{17}F_3N_4O$) C, H, N.

3-(4-Fluoro-phenyl)-5-methyl-4,5-dihydro-isoxazole-5-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide 9f. 4-Fluorobenzamidoxime (1.39 g, 10 mmol) was mixed with triethylamine (200 mg, 2.0 mmol) and NaOCl (4%, 15 mL, 10 mmol) in CH_2Cl_2 (25 mL) at 0 °C over 5 min. *N*-(4-(Cyano-3-trifluoromethyl-phenyl)-2-methyl-acrylamide **5b** (508 mg, 2.0 mmol) was added into the mixture, and the mixture was warmed to room temperature and then stirred for 3 h at room temperature. The reaction mixture was quenched by $NaHCO_3$ and then extracted with ethyl acetate. The organic layer was combined, washed with brine, dried over Na_2SO_4, and concentrated to yield a crude product. Purification of the crude product on CombiFlash system using hexanes and ethyl acetate from pure hexanes to 1:1 to yield the title compound as a white solid (2.54 g). Yield 65%. ^{1}H NMR ($CDCl_3$) δ 9.15 (s, 1H), 8.15 (s, 1H), 7.85 (dd, J = 6.5, 1.2 Hz, 2H), 7.60 (m, 2H), 7.05 (m, 2H), 3.75 (abq, J = 12.4 Hz, 2 H), 1.75 (s, 3H). MS (*m*/*z*): 392, $(M + H)^+$. Anal. ($C_{19}H_{13}F_4N_3O_2 \cdot 0.5$MeOH) C, H, N.

3,5-Dimethyl-4,5-dihydro-isoxazole-5-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide 9e. Compound **9e** was prepared from coupling acetaldehyde oxime and **5b** according to the procedure used to prepare **9f**. Yield 52%. ^{1}H NMR ($CDCl_3$) δ 8.65 (s, 1H), 7.95 (s, 1H), 7.40 (m, 2H), 3.40 and 2.95 (abq, J = 14.0 Hz, 2H), 2.00 (s, 3H), 1.70 (s, 3H). MS (*m*/*z*): 312, $(M + H)^+$. Anal. ($C_{14}H_{12}F_3N_3O_2 \cdot 1.4H_2O$) C, H, N.

3-Methyl-2-(2,2,2-trifluoro-acetyl)-5-trifluoromethyl-3,4-dihydro-2*H*-pyraz ole-3-carboxylic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide 9g. 3-Methyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic acid (4-cyano-3-trifluoromethyl-phenyl)-amide **7c** (546 mg, 1.5 mmol) in DCM (∼ 20 mL) was treated with pyridine (162 μL, 2.0 mmol) followed by trifluoroacetic anhydride (209 μL, 1.5 mmol) dropwise at 0 °C. The reaction was stirred for another 2 h. The solvent was removed, and the residue was partitioned between DCM and water. The organic layer was washed with sat. sodium bicarbonate, water, and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated to give the crude material, which was then purified by CombiFlash system (silica gel, hexanes:ethyl acetate from 10:1 to 2:1) to yield the title compound as off-white solid (586 mg). Yield, 85%. ^{1}H NMR ($CDCl_3$) δ 9.72 (s, 1H), 8.08 (s, 1H), 7.88 (d, J = 8.5 Hz, 1H), 7.80 (d, J = 8.5 Hz, 1H), 4.35 (abq, J = 12.5 Hz, 1H), 3.10 (abq, J = 12.5 Hz, 1H), 2.01 (s, 3H). MS (*m*/*z*): 461, $(M + H)^+$. Anal. ($C_{16}H_9F_9N_4O_2$) C, H, N.

3-Methyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carbothioic Acid (4-Cyano-3-trifluoromethyl-phenyl)-amide 10c. 3-Methyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboxylic acid (4-cyano-3-trifluoromethyl-phenyl)-amide **7c** (5.46 g, 15 mmol) and Lawesson's reagent (6.07 g, 15 mmol) in toluene (100 mL) were refluxed for 6 h until the solution turned clear. The reaction was cooled down, and some precipitate was observed. The solid was removed by filtration, and the filtrate was concentrated to give the crude product as a green oil, which was then purified by Combiflash system (DCM:ethyl acetate as eluent, from pure DCM to 1:1) to afford the title compound as a green solid (1.82 g). Yield, 32%.^{1}H NMR ($CDCl_3$) δ 11.05 (s, 1H), 8.45 (s, 1H), 8.30 (d, J = 8.5 Hz,1H), 7.82 (d, J = 8.5 Hz, 1H), 5.95 (br, s, 1H), 3.40 (abq, J = 9.5 Hz, 1H), 3.21 (abq, J = 9.5 Hz, 1H), 1.85 (s, 3H). MS (*m*/*z*): 381, $(M + H)^+$.

***N*-(4-Cyano-3-trifluoromethyl-phenyl)-2,3-dimethyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboximidic Acid Methyl Ester 10a. 10c** (3.80 g, 10 mmoL), K_2CO_3 (2.07 g, 15 mmoL) in acetone (10 mL) was treated with EtI (800 μL, 10 mmoL) at room temperature. The reaction was slightly heated and stirred at 50 °C for 1 h. The solid was filtrated, and the filtrate was concentrated to give the crude product as a brown oil, which was then purified by

CombiFlash system with a silica gel cartridge using hexanes and ethyl acetate as eluent to afford *N*-(4-cyano-3-trifluoromethyl-phenyl)-3-methyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboximidothioic acid ethyl ester as a colorless oil (3.3 g). Yield 81%. ^{1}H NMR ($CDCl_3$) δ 7.80 (d, J = 7.8 Hz, 1H), 7.28 (s, 1H), 7.15 (d, J = 7.9 Hz, 1H), 6.50 (s, 1H), 3.51 (abq, J = 12.5 Hz, 1H), 2.88 (abq, J = 12.5 Hz, 1H), 2.35 (q, J = 8.5 Hz, 2H), 1.08 (t, J = 8.5 Hz, 3H). MS (*m*/*z*): 409, $(M + H)^+$.

N-(4-Cyano-3-trifluoromethyl-phenyl)-3-methyl-5-trifluoromethyl-3,4-dihydro-2*H*-pyrazole-3-carboximidothioic acid ethyl ester (490 mg, 1.2 mmol) in dioxane (10 mL) was treated with MeOH (10 mL) in a sealed tube. The reaction mixture was heated to 100 °C for 4 h. The solvent was removed, and the residue was purified by CombiFlash system (silica gel, ethyl acetate and methanol as eluent, from pure ethyl acetate to 4:1) to afford the title compounds as a white solid (193 mg). Yield 41%. ^{1}H NMR ($CDCl_3$) δ 8.21 (s, 1H), 8.05 (d, J = 8.0 Hz, 1H), 7.90 (d, J = 8.0 Hz, 1H), 4.36 (s, 3H), 3.52 (s, 3H), 3.36 (d, J = 12.5 Hz, 1H), 3.10 (d, J = 12.5 Hz, 1H), 1.52 (s, 3H). MS (*m*/*z*): 393, $(M + H)^+$. Anal. ($C_{14}H_{10}F_6N_4O$) C, H, N.

(*R*)-12. The compound was prepared by the procedure reported in our recent communication.[15]

(*R*)-13. (*R*)-**12** (1.32 g, 3.4 mmoL), 4-iodo-phenyl isocynate (780 mg, 3.2 mmoL) in dioxane (10 mL) was heated to 100 °C for 1 h. The solvent was removed, and the residue was purified by CombiFlash system using hexanes: ethyl acetate 6: 1 to 1:1 as eluent to afford the title compound as a white solid, which was recrystallized from DCM for X-ray crystallography determination (1.16 g). Yield 54%. ^{1}H NMR ($CDCl_3$) δ 10.35 (s, 1H), 7.82 (d, J = 7.0 Hz, 1H), 7.58 (d, J = 8.5 Hz, 2H), 7.52 (s, 1H), 7.35 (d, J = 7.5 Hz, 1H), 6.89 (d, J = 8.5 Hz, 1H), 3.55 (abq, J = 11.5 Hz, 1H), 3.12 (abq, J = 11.5 Hz, 1H), 1.72 (s, 3H). MS (*m*/*z*): 634, $(M + H)^+$.

X-ray Crystal Structure Determination. Crystals of (*R*)-**13** were grown from evaporating CH_2Cl_2. A full hemisphere of diffracted intensities (1868 20-s frames with an omega scan width of 0.30°) was measured using graphite-monochromated Mo Kα radiation on a Bruker SMART APEX CCD single-crystal diffraction System. X-rays were provided by a fine-focus sealed X-ray tube operated at 50 kV and 35 mA. Lattice constants were determined with the Bruker SAINT software package using peak centers for 1384 reflections. A total of 16403 integrated reflection intensities having 2Θ(Mo K) < 46.51° were produced using the Bruker program SAINT; 13647 of these were unique and gave $R_{int} = 0.080$. The data were corrected empirically for variable absorption effects using 143 equiv reflections. The Bruker SHELXTL-PC software package was used to solve the structure using "direct methods" techniques. All stages of weighted full-matrix least-squares refinement were conducted using F_o^2 data with the SHELXTL-PC Version 6.12 software package. Final agreement factors at convergence are: R_1(unweighted, based on F) = 0.068 for 6542 independent absorption-corrected reflections having 2Θ(Mo K) < 46.51° and I > $2\sigma(I)$; R_1(unweighted, based on F) = 0.127 and wR_2 (weighted, based on F^2) = 0.158 for all 13647 independent absorption-corrected reflections having 2Θ(Mo K) < 46.51°. The absolute configuration was determined experimentally using anomalous dispersion of the X-rays. The "Flack" absolute structure parameter refined to a final value of −0.03(3).

The structural model incorporated anisotropic thermal parameters for all nonhydrogen atoms and isotropic thermal parameters for all hydrogen atoms. The eight hydrogens bonded to nitrogen were initially located from a difference Fourier synthesis. They were then included in the structure factor calculations as idealized atoms (assuming sp^2-hybridization of the nitrogen atoms and a N−H bond length of 0.88 Å) "riding" on their respective nitrogen atoms. The four methyl groups (C_{15}, C_{45}, C_{75}, C_{105} and their hydrogens) were refined as rigid rotors (using idealized sp^3-hybridized geometry and a C−H bond length of 0.98 Å) which were allowed to rotate around their respective C−C bonds in least-squares cycles. The remaining hydrogen atoms were included in the structure factor calculations as idealized atoms (assuming sp^2- or sp^3-hybridization of the carbon atoms and C−H bond lengths of 0.95−0.99 Å) "riding" on their respective carbon atoms. The isotropic thermal parameters of all hydrogen atoms were fixed at values 1.2 (nonmethyl) or 1.5 (methyl) times the equivalent isotropic thermal parameter of the carbon or nitrogen atom to which they are covalently bonded. Moderate restraints had to be applied to the anisotropic thermal parameters of 18 nonhydrogen atoms.

Biological Evaluation. Immature Rat Ventral Prostate and *Levator ani* Weight in Vivo Assay. Immature (approximately 50 g) castrated male Sprague Dawley rats (Charles River) were treated once daily for 5 days with test compound (usually given orally at 2 mg/d in a volume of 0.3 mL, in 20% cyclodextrin or 0.5% methylcellulose vehicle), or with testosterone propionate (administered subcutaneously by injection at the nape of the neck at 1 mg/d, in a volume of 0.1 mL in sesame oil), or with vehicle (1 mL of 20% cyclodextrin or 0.5% methylcellulose, given orally). On the sixth day, the rats were euthanized by asphyxiation in carbon dioxide. Ventral prostates and *levator ani* muscles were removed and their wet weights determined. Test compound activity was determined as the percent stimulation of tissue weight, with the vehicle-treated control group set to zero percent and the testosterone alone-treated control group set to 100%.

Immature Rat Ventral Prostate Weight in Vivo Assay. Immature (approximately 50 g) castrated male Sprague Dawley rats (Charles River) were treated once daily for 5 days with test compound (usually given orally at 2 mg/d in a volume of 0.3 mL, in 30% cyclodextrin or 0.5% methylcellulose vehicle) and with testosterone propionate (given subcutaneously by injection at the nape of the neck at 0.1 mg/d, in a volume of 0.1 mL in sesame oil), or with vehicle (1 mL of 20% cyclodextrin or 0.5% methylcellulose, given orally). On the sixth day, the rats were euthanized by asphyxiation in carbon dioxide. Ventral prostates were removed and their wet weights determined. Test compound activity was determined as the percent inhibition of testosterone-enhanced tissue weights, with a vehicle-treated control group set to 100% and a testosterone alone-treated control group set to 0%.

Mature Rat Ventral Prostate and *Levator ani* Weight in Vivo Assay. Mature (150 to 200 g) castrated male Sprague Dawley rats (Charles River) were treated once daily for 14 days with test compound (usually administered by oral gavage at up to the desired dosage, up to 30 mg/kg in a volume of 1 mL, in 20% cyclodextrin or 0.5% methylcellulose vehicle), or with testosterone propionate (administered subcutaneously by injection at the nape of the neck at 5 mg/kg, in a volume of 0.1 mL in sesame oil), or with vehicle (1 mL of 20% cyclodextrin or 0.5% methylcellulose, given orally). On the fifteenth day, the rats were euthanized by asphyxiation in carbon dioxide. Ventral prostates and *levator ani* muscles were removed and their wet weights determined. Test compound activity was determined as the percent stimulation of tissue weight, with the vehicle-treated control group set to zero percent and the testosterone alone-treated control group set to 100%.

Mature Rat Ventral Prostate Weight in Vivo Assay. Mature (150 to 200 g) castrated male Sprague Dawley rats (Charles River) were treated once daily for 6 weeks with test compound (usually administered by oral gavage at up to the desired dosage, up to 30 mg/kg in a volume of 1 mL, in 20% cyclodextrin or 0.5% methylcellulose vehicle) and with testosterone propionate (administered subcutaneously by injection at the nape of the neck at 5 mg/kg, in a volume of 0.1 mL in sesame oil), or with vehicle (1 mL of 20% cyclodextrin or 0.5% methylcellulose, given orally). At the end of 6 weeks, the rats were euthanized by asphyxiation in carbon dioxide. Ventral prostates were removed and their wet weights determined. *Levator ani* muscles were also removed and their weight were determined. Test compound activity was determined as the percent inhibition of testosterone-enhanced tissue weights, with a vehicle-treated control group set to 100% and a testosterone alone-treated control group set to 0%.

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Supporting Information Available: X-ray crystallographic data on (*R*)-**13**, results from elemental analysis or HPLC analysis for all tested compounds, procedure and spectra data of substituted anilines. This material is available free of charge via the Internet at http://pubs.acs.org.

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