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Synthesis and SAR of amino acid-derived heterocyclic progesterone receptor full and partial agonists

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ABSTRACT

Two classes of amino acid-derived heterocyclic progesterone receptor ligands were developed to address the metabolic issues posed by the dimethyl amide functionality of the lead compound (**1**). The tetrazole-derived ligands behaved as potent partial agonists, while the 1,2,4-triazole ligands behaved as potent full agonists.

The steroid hormones progesterone (P4) and estrogen (E2) play an important role in the female reproductive process. In the uterus, E2 stimulates the proliferation of the endometrium, while P4 blocks this growth in an E2-dependent manner.[1] While the exact mechanism of this effect is not fully understood, in vitro and in vivo studies have demonstrated that this antagonism requires complexation of P4 with the progesterone receptor (PR)[2] and can operate through two general pathways: (1) an autocrine mechanism in which liganded PR interferes with the ability of E2-bound estrogen receptor (ER) to stimulate gene expression,[3] and (2) a paracrine mechanism whereby P4-bound PR in one cell type, through its gene products, suppresses E2-stimulated gene expression in another cell type.[4]

In addition to regulating the female reproductive cycle, P4 and E2 also play a role in modulating endometriosis, a condition characterized by growth of endometrial tissue outside of the uterus that is associated with dysmenorrhea, chronic pelvic pain, fatigue and a host of other symptoms. As with endometrial tissue within the uterus, proliferation of endometrial lesions is stimulated by E2 and blocked by P4.[5] Consequently, treatments for endometriosis include the use of P4 and other progestins to moderate growth of the offending lesions. Progestin therapies have been found to be efficacious, but not without unpleasant side effects that include depression, breakthrough bleeding and breast tenderness, some of which are mediated through PR agonism.[6] Additional adverse effects may be attributed to the poor nuclear hormone receptor (NHR) selectivity of most progestins, including P4 itself.[7]

In the search for improved endometriosis treatments, opportunity exists for exploitation of the diverse pathways involved in E2 opposition by PR agonism, including the development of small molecule PR modulators, PR partial agonists, and highly selective PR full agonists. A PR modulator strategy would, via selective recruitment of cofactors by the liganded PR, be dependent upon selective expression of proteins responsible for mediating E2 opposition while avoiding expression of proteins that mediate adverse effects.[8] Alternatively, a PR partial agonist approach would not rely on differential cofactor recruitment, but on reducing the level of PR-regulated gene expression such that enough gene products are obtained to oppose E2 but not to induce side effects. Finally, a highly selective PR full agonist would offer E2 antagonism without the side effects brought about by activity at other NHRs. The work disclosed herein is the result of efforts applied to the latter two approaches.

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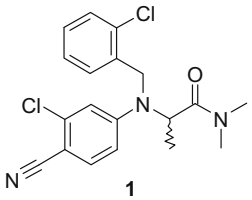
Specifically, the goal of this program has been to identify small molecules having a profile that includes potent binding at the progesterone receptor (ideally <20 nM), ⩾100-fold selectivity over other NHRs, including the androgen receptor (AR), glucocorticoid receptor (GR), mineralocorticoid receptor (MR) and ER, and partial-to-full PR agonism. Chemistry initiated with the lead dimethyl amide **1** (Fig. 1). Compound **1** demonstrated a PR binding IC_{50} of 32 nM[9] and sub-nanomolar activity in a T47D-based alkaline phosphatase PR agonist assay.[10,11] With respect to NHR selectivity, compound **1** was 50-fold selective over AR and >100-fold selective over GR, MR and ER, indicating that AR selectivity required improvement. Early in the team's efforts to optimize the pharmacological properties of **1**, it became apparent that the dimethylamide group was metabolically unstable, and isosteric replacement of this moiety might offer a means of discharging this liability.

Applying the strategy of utilizing five-membered ring heterocycles as amide surrogates, a survey of heterocyclic amide replacements was conducted. A number of ligands were identified as having good to excellent PR binding potency and agonist activity in the T47D PR agonist assay (Table 1). Across all heterocycles examined, P450 inhibition profiles (represented by inhibition at the 2C19 isoform in Table 1), would require optimization and offered no means of differentiation. Examination of the binding and functional parameters, however, did allow for differentiation, as those ligands with a methyl substituent in the 2-position relative to the point of core attachment, (exemplified by entries **4**, **7**, **9** and **10**) were found to be both more selective over AR and significantly more potent in the agonist assay. The reduced cell potencies of compounds **3**, **5**, **6**, and **8** coupled with the lower AR selectivity of compounds **3**, **5**, and **6** rendered them less appealing. Based on their exceptional potencies and selectivities, tetrazole **9** and triazole **10** were selected for further SAR activities.

Analogs of **9** were prepared as shown in Scheme 1.[12] Reaction of 2-chloro-4-fluorobenzonitrile with an amino acid followed by Fischer esterification produced ester **11**. Treatment with an aluminum amide was followed by reaction with TMS azide under Mitsunobu-like conditions[13] to afford tetrazole **12**, which was then alkylated to provide target tetrazoles **9** and **13–22**. In most cases, the syntheses began with single amino acid enantiomers, however it was later determined that scrambling of the stereocenter bearing the R^2 substituent occurred during alkylation of the aniline nitrogen. In the cases examined, the racemization was not complete, and with the exception of the example in which the enantiomers were separated (vide infra), compounds were tested as mixtures of undetermined ee and are shown without indication of absolute stereochemistry.

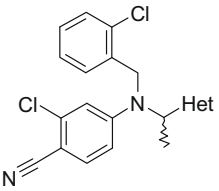
Several SAR trends were identified in this class of tetrazoles. For example, substitution at the R^2 position was important for potency in the PR functional assay (Table 2, cf. compound **13** vs. compounds **9**, **14**, and **15**). In addition, while all other compounds exemplified in Table 2 were at least 100-fold selective for PR over AR, compound **13** was only 40-fold selective (data not shown). Increasing the length of the R^2 substituent led to an attenuation in intrinsic agonism (and potency to a lesser extent) such that compound **15**

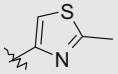
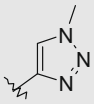
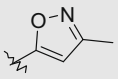
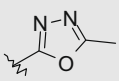


1
PR IC_{50} = 32 nM
T47D EC_{50} = 0.7 nM

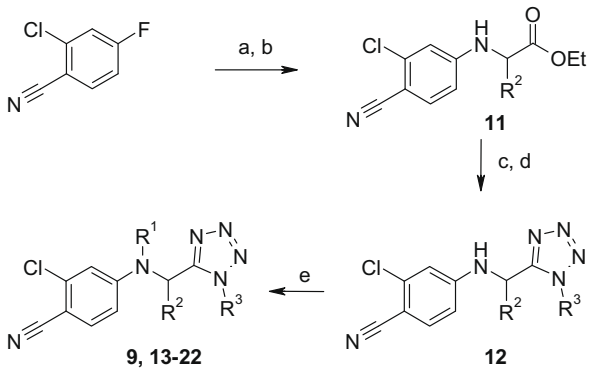
Figure 1. Lead PR ligand **1**.

Table 1
Selected in vitro data for compounds **3–10**.[a]



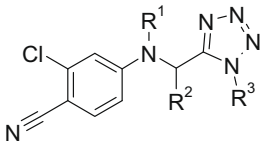
Compd	Het	PR binding IC_{50} (nM)	AR binding selectivity (-fold)	PR T47D EC_{50} (nM) (% P4)	P450 2C19 inhibition IC_{50} (μM)
3		13[b]	15	380 (19)	0.067
4		25	318	6.0 (102)	<0.033
5		40	16	606 (32)	0.040
6		20	10	272 (22)	<0.033
7		20	500	0.60 (99)	<0.033
8		32	163	14 (80)	0.091
9		13[b]	>770	0.02 (92)	<0.033
10		13[b]	>770	0.5 (107)	<0.033

[a] Values are the mean of ⩾2 determinations.
[b] Tight-binding limit of the assay.



Scheme 1. Reagents and conditions: (a) $H_2NCH(R^2)CO_2H$, K_2CO_3, DMF/H_2O, 90 °C, 15 h; (b) EtOH, H_2SO_4, 80 °C, 15 h; (c) R^3NH_2·HCl, $AlMe_3$, $PhCH_3$, 0–60 °C, 36 h; (d) $TMSN_3$, PPh_3, DIAD, THF, 0–23 °C, 4d; (e) R^1Br, NaH, DMF, 0–23 °C, 15 h.

Table 2
Selected in vitro data for compounds **9** and **13–22**[a]

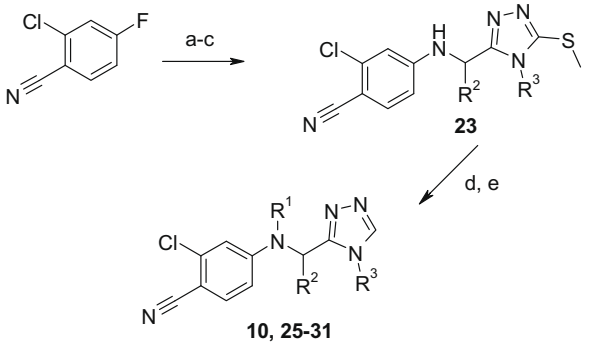


Compd	R^1	R^2	R^3	PR IC_{50} (nM)	T47D EC_{50} (nM) (% P4)	P450 2C19 IC_{50} (μM)
9	2-Cl–Bn	Me	Me	13	0.02 (93)	<0.033
13	2-Cl–Bn	H	Me	50	197 (ND)	<0.33
14	2-Cl–Bn	Et	Me	13	0.04 (73)	<0.033
15	2-Cl–Bn	*i*-Bu	Me	5	1.3 (60)	0.30
16	2-Cl–Bn	Me	Et	16	0.6 (122)	<0.033
17	2-Cl–Bn	Me	*i*-Pr	32	35 (116)	0.08
18	2-CF_3–Bn	*i*-Bu	Me	25	139 (56)	1.3
19	*i*-Bu	*i*-Bu	Me	16	9.5 (47)	8.8
20	*c*-Bu(CH_2)	*i*-Bu	Me	40	11 (31)	17
21	*c*-Bu(CH_2)	(*S*)-*i*-Bu[b]	Me	16	6.8 (38)	6.4
22	*c*-Bu(CH_2)	(*R*)-*i*-Bu[b]	Me	200	ND	ND

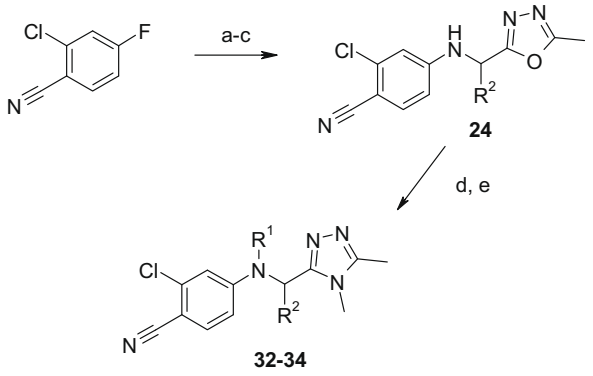
[a] Values are means of ⩾ 2 determinations.
[b] See Ref. 15. ND = Not determined.

demonstrated intrinsic agonism that was 60% of P4. By contrast, adding steric bulk directly to the tetrazole ring resulted in decreased agonist potency, but not intrinsic agonism (Table 2, cf. compound **9** vs. compounds **16** and **17**). The P450 inhibition (represented by 2C19 inhibition) was most influenced by modification of the R^1 aniline substituent. Compound **18**, in which the 2-chlorobenzyl substituent was replaced with a 2-(trifluoromethyl)benzyl substituent, demonstrated markedly reduced 2C19 inhibition, but this improvement appeared to come at the cost of agonist potency. Ultimately, replacement of benzyl groups at R^1 with aliphatic substituents led to the identification of partial agonists **19** and **20**, each having excellent PR binding, functional potency, and PR partial agonism. Compound **20** was separated into its individual enantiomers **21** and **22** and all PR agonist activity was found to reside in enantiomer **21**.[14]

Synthesis of analogs of **10** began with the same amino acid—aryl fluoride displacement reaction as for the tetrazole class (Scheme 2).[15] Coupling of the product acid with 4-methyl-3-thiosemicarbazide followed by a one-pot cyclization/alkylation[16] provided methylthio-substituted intermediate **23**. Alkylation of the aniline nitrogen and reductive removal of the methylthio group afforded target triazoles **10** and **25–31**. An additional set of compounds in which a methyl group was incorporated at the five-position of the triazole ring was prepared as shown in Scheme 3.[17]



Scheme 2. Reagents and conditions: (a) $H_2NCH(R^2)CO_2H$, K_2CO_3, DMF/H_2O, 90 °C, 15 h; (b) 4-methyl-3-thiosemicarbazide, EDC, *i*-Pr_2NEt, HOBT, THF, 23 °C, 24 h; (c) NaOMe, MeOH, 60 °C, 15 h, then MeI, 24 h; (d) RaNi, EtOH, 80 °C, 15 h; (e) NaH, DMF, R^1Br, 0–23 °C, 15 h.



Scheme 3. Reagents and conditions: (a) $H_2NCH(R^2)CO_2H$, K_2CO_3, DMF/H_2O, 90 °C, 15 h; acetylhydrazide, EDC, HOBT, THF, 23 °C, 18 h; (c) $POCl_3$, CH_3CN, 75 °C, 3 h; (d) CH_3NH_2, MeOH, microwave, 200 °C, 1 h; (e) NaH, DMF, R^1Br, 0–23 °C, 15 h.

Amino acid—aryl fluoride displacement was followed by coupling with acetylhydrazide and cyclization to oxadiazole **24**. Conversion of the oxadiazole to the *N*-methyl-1,2,4-triazole could be accomplished by heating in a microwave in the presence of an excess of methylamine in methanol.[18] Finally, alkylation of the aniline nitrogen produced target triazoles **32–34**. As with the tetrazole class, some scrambling of the stereocenter adjacent to R^2 occurred during the aniline alkylation. Compounds were tested as mixtures of undetermined ee and are shown without indication of absolute stereochemistry.

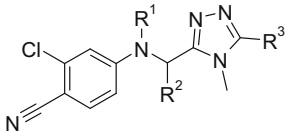
Triazole-based PR ligands were similar in potency to their tetrazole-based counterparts, and several of the same SAR trends were observed. As seen with entries **10** and **25–28** (Table 4), increasing the size of the substituent in the R^2 position attenuated intrinsic agonism but also produced a corresponding drop in potency. As with the tetrazole class, P450 inhibition (represented by 2C19 inhibition) was observed with most analogs, and although modifications at R^1 provided the same benefit, a significant drop in progesterone-related potencies was observed (cf. **18** vs **29** and **19** vs **30**). The exception to this was cyclohexylmethylene-substituted analog **31**, which had good PR binding and functional potency, full agonism, and an acceptable P450 inhibition profile. Another strategy for reducing P450 inhibition was introduction of a methyl group to the five-position of the triazole, exemplified by compounds **32–34**. While compound **34**, with significant bulk at R^2,

Table 3
Rat PK for compounds **19** and **10**[a]

Parameter	Compound	
	19[b]	**10**[c]
Dose (iv, po, mg/kg)	1.1, 2.0	1.2, 2.3
CLp (mL/min/kg)	Very high[e]	4.85 (1.15)
Vd_{ss} (L/kg)	Very large[e]	0.54 (0.08)
Oral C_{max} (ng/mL)	146 (117)	2116 (583)
Oral AUC_{0-t}, (h ng/mL)	572 (67)	10085 (1779)
$t_{½}$, po (h)	8.9 (0.7)	2.4 (0.3)
Oral%F	~100	~100

[a] Values are means of three experiments, standard deviation is given in parentheses.
[b] Discrete study.
[c] Cassette study conducted as a mixture of four compounds.
[e] Unable to quantitate.

Table 4
Selected in vitro data for compounds **10** and **25–34**[a]



Compd	R^1	R^2	R^3	PR IC_{50} (nM)	PR T47D EC_{50} (nM) (% P4)	2C19 IC_{50} (μM)
10	2-Cl–Bn	Me	H	13	0.5 (107)	<0.033
25	2-Cl–Bn	Et	H	16	0.02 (97)	<0.33
26	2-Cl–Bn	*n*-Pr	H	13	0.05 (84)	0.11
27	2-Cl–Bn	*i*-Pr	H	32	4.7 (76)	0.063
28	2-Cl–Bn	*i*-Bu	H	25	157 (58)	1.3
29	2-CF_3–Bn	*i*-Bu	H	398	ND	1.5
30	*i*-Bu	*i*-Bu	H	500	ND	ND
31	*c*-Hex(CH_2)	Me	H	40	3.0 (140)	3.8
32	2-Cl–Bn	Me	Me	63	4.5 (119)	43
33	2-Cl–Bn	*n*-Pr	Me	50	4.0 (87)	4.1
34	2-Cl–Bn	*i*-Pr	Me	398	1665 (61)	ND

[a] Values are the means of ⩾2 determinations. ND = not determined.

suffered from markedly reduced PR binding and functional potency, analogs **32** and **33** were free of potent P450 inhibition and were found to be single-digit nanomolar full agonists of PR.

The pharmacokinetic parameters of tetrazole **19** and triazole **10** were evaluated in rats (Table 3). Plasma clearance for **19** was found to be too high to quantitate, likely driven by a large volume of distribution that was likewise too large to quantitate. By comparison, triazole **10** demonstrated low plasma clearance and a smaller volume of distribution. In addition, the oral AUC of **10** was approximately 18-fold higher than that of tetrazole **19**. The oral half-life of tetrazole **19**, perhaps due to its large volume of distribution, was nearly 9 h, while that of triazole **10** was 2.4 h, suggesting that, even though the overall exposure of **10** was greater, both compounds would be suitable for in vivo studies.

In summary, replacement of the dimethyl amide of lead PR ligand **1** with 5-membered ring heterocycles led to the discovery of two series of selective PR partial and full agonists. The tetrazole class, when optimized for potency, selectivity and P450 inhibition, produced PR ligands with in vitro partial agonist profiles, while efforts in the 1,2,4-triazole class allowed for the identification of potent full agonists. Rat PK studies with compounds from both classes indicated that the overall exposure of triazole **10** was more than 15-fold greater than that for tetrazole **19**. Conversely, the oral half life of **19** was nearly fourfold longer, indicating that both compounds might be suitable for in vivo pharmacology studies. Results of these studies will be reported elsewhere.

Acknowledgments

The authors would like to thank William Leister for the chiral separation of compound **20** and our colleagues in the Screening and Compound Profiling group for performing the PR and AR binding assays. We would also like to thank Dennis A. Holt for many helpful discussions.

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9. The PR binding assay was performed according to the manufacturers protocol (PR Competitor Assay Kit, Red—(Invitrogen–Product No. P2962)) with minor amendments. Briefly, 40 nM PR-Ligand Binding Domain, 2 nM Fluormone PL Red and 1 mM DTT were dissolved and mixed in Complete PR RED Buffer supplemented with 2 mM CHAPS. 10 μL of the mix was dispensed to each well of Greiner low volume plates, containing compounds at the required concentration. The plates were spun for 1 min at 200 g, covered to protect the reagents from light, and then incubated at room temperature for approximately 2 h. Plates were read on an Acquest using a 530–25 nm excitation and 580–10 nm emission interference filter and a 561 nm dichroic mirror.
10. The T47D alkaline phosphatase PR agonist assay was performed in accordance to procedures set forth in the literature. In short, 120 μL of T47D cell suspension was seeded into a 96-well plate and allowed to attach to the plate overnight. On the next day, the cells were treated with compound and incubated overnight. On the following day, 100 μL of pNPP-SPAP is added and were allowed to stand in the dark for 2 h. Optical density is then measured at a wavelength of 405 nm on a plate reader.
11. Di Lorenzo, D.; Albertini, A.; Zava, D. *Cancer Res.* **1991**, *51*, 4470–4475.
12. *Example preparation*: compound **9**. Step 1: A suspension of 2-chloro-4-fluorobenzonitrile (8 g, 51.4 mmol), L-alanine (5.5 g, 67.7 mmol), and K_3CO_3 (10.66 g, 77.1 mmol) in DMF (260 mL) and H_2O (40 mL) were heated with stirring at 90 °C for 15 h. Water (200 mL) was added and the reaction mixture was acidified to pH 4 with 6 N HCl and extracted with EtOAc. The organic extracts were dried over Na_2SO_4 and concentrated. The crude product was used in the next step without purification. Step 2: The crude aniline was taken up in a solution of EtOH (150 mL) and H_2SO_4 (8 mL) and the solution was stirred at 80 °C for 24 h. The solvent was removed and the residue was neutralized with saturated $NaHCO_3$ and extracted with Et_2O. The organic layer was washed with H_2O, dried over Na_2SO_4 and concentrated. The residue was purified via silica gel chromatography with 5–20% EtOAc/hexane to yield the ester (8.42 g, 65%) as a light yellow oil. Step 3: Trimethylaluminum (2 M in toluene, 9.6 mL, 19.2 mmol) was added slowly to a suspension of methylamine hydrochloride (1.3 g, 19.2 mmol) in toluene (19 mL) at 0 °C. After warming to rt, a solution of the ester (907 mg, 3.84 mmol) in toluene (6 mL) was added and the reaction solution was stirred at 60 °C for 16 h. After cooling to 0 °C, 1 N HCl (20 mL) was added. After stirring for 15 min, saturated $NaHCO_3$ and brine were added and the mixture was extracted with EtOAc. The organic extracts were dried over Na_2SO_4 and concentrated. The solid residue was suspended in Et_2O and collected by filtration to afford the amide as a beige solid (490 mg, 54%). Step 4: DIAD (0.77 mL, 3.95 mmol) and $TMSN_3$ (1.05 mL, 7.92 mmol) were added alternatingly to a solution of the amide (470 mg, 1.98 mmol) and PPh_3 (1.04 g, 3.95 mmol) in a mixture of THF (10 mL) and acetonitrile (10 mL) at 0 °C. After 3 days at rt, the reaction mixture was diluted with H_2O and extracted with EtOAc. The organics were dried over Na_2SO_4 and concentrated. The residue was purified using silica gel chromatography with 20–50% EtOAc/hexane to give the tetrazole (630 mg, 120% (contaminated with PPh_3O)) as a light yellow solid. Step 5: Sodium Hydride (60% in mineral oil, 23 mg, 0.57 mmol) was added to a solution of the tetrazole (100 mg, 0.38 mmol) in DMF (4 mL) at 0 °C. After

stirring for 30 min, 1-(bromomethyl)-2-chlorobenzene (117 mg, 0.57 mmol) was added and the reaction mixture was stirred at rt for 1 h, quenched with water and extracted with EtOAc. The organic layer was washed with H_2O, dried over Na_2SO_4 and concentrated. The residue was purified via silica gel chromatography with 5–50% EtOAc/hexane to give the pure tetrazole (83 mg, 53%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 7.49 (d, 1H, J = 8.8 Hz), 7.39 (d, 1H, J = 9.2 Hz), 7.22 (t, 1H, J = 7.6 Hz), 7.15 (t, 1H, J = 7.6 Hz), 6.89 (m, 2H), 6.66 (dd, 1H, J = 8, 4 Hz), 5.46 (dd, 1H, J = 12, 8 Hz), 4.72 (d, 1H, J = 20 Hz), 4.50 (d, 1H, J = 20 Hz), 3.97 (s, 3H), 1.88 (d, 3H, J = 4 Hz). LC–MS (ES) m/z 386.8 $(M+H)^+$.

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14. The absolute configuration for compound **22** was determined by ab initio VCD analysis. The confidence limit for this assignment was estimated to be 88%, using linear regression analysis to correlate observed and experimental VCD intensities for a set of thirteen marker bands. The calculated IR spectrum was in very good overall agreement with the experimental spectrum, indicating good modeling of the conformational space (required for reliable VCD assignments). Based on these results, the absolute configuration assigned to compound **22** was considered to be reliable.
15. *Example preparation*: compound **10**. Step 1: Conducted as described in Ref. 12, Step 1. Step 2: *N*-methylhydrazinecarbothioamide (3.38 g, 32.1 mmol), HOBT (3.47 g, 25.7 mmol) and EDC (6.15 g, 32.1 mmol) were added to a solution of *N*-(3-chloro-4-cyanophenyl)-L-alanine (5.77 g, 25.7 mmol) in DMF (20 mL) and THF (100 mL). After stirring for 24 h, EtOAc was added and the reaction mixture was washed with aq $NaHCO_3$, brine and H_2O. The organics were dried over Na_2SO_4, concentrated, and purified via silica gel chromatography with 4–10% MeOH/CH_2Cl_2 to give the product (3.19 g, 40%) as a white solid. Step 3: Sodium methoxide (0.83 g, 15.3 mmol) was added to a mixture of the product from Step 2 (3.19 g, 10.2 mmol) in MeOH (100 mL) and the reaction mixture was stirred at 60 °C for 15 h. After cooling, MeI (1.27 mL, 20.4 mmol) was added and the reaction mixture was stirred at room temperature for 24 h. The solvent was removed and the residue was partitioned between EtOAc and H_2O. The organic layer was washed with H_2O and concentrated. The resulting light yellow solid was triturated with EtOAc to give the product (2.17 g, 69%) as a white solid. Step 4: Raney-nickel (excess) was added to a suspension of the (methylthio)triazole (2.17 g, 7.05 mmol) in EtOH (71 mL) and the reaction mixture was stirred at 80 °C for 15 h. The reaction mixture was filtered through a pad of Celite and concentrated. The residue was purified via silica gel chromatography with 0.5–5% MeOH/CH_2Cl_2 to give the product (1.18 g, 64%) as a light yellow solid. Step 5: Conducted as described in Ref. 12, Step 5 to afford triazole **10** as a light yellow solid (25 mg, 33%). ^{1}H NMR (CD_3OD) δ 8.31 (s, 1H), 7.56 (d, 1H, J = 8.8 Hz), 7.35 (d, 1H, J = 7.2 Hz), 7.18 (m, 2H), 7.10 (s, 1H), 6.92 (m, 2H), 5.78 (m, 1H), 4.82 (d, 1H, J = 18.4 Hz), 4.45 (d, 1H, J = 18.4 Hz), 3.64 (s, 3H), 1.76 (d, 3H J = 6.4 Hz). LCMS (ES) m/z 386.2 $(M+H)^+$.
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17. *Example preparation*: compound **34**. Step 1: Conducted as described in Ref. 12, Step 1 using L-valine in place of L-alanine. Step 2: EDC (9.29 g, 48.5 mmol) was added in one portion to a solution of the aniline (9.8 g, 38.8 mmol), acetylhydrazide (3.59 g, 48.5 mmol) and HOBT (5.94 g, 38.8 mmol). The resultant mixture was stirred at rt for 18 h, diluted with water and extracted with EtOAc. The organics were washed with brine, dried over Na_2SO_4 and concentrated. The residue was purified by silica gel chromatography using 0–10% MeOH/CH_2Cl_2 to afford the bis-acylhydrazide (4.84 g, 40%). Step 3: $POCl_3$ (7.25 mL, 78 mmol) was added via syringe to a solution of the bis-acylhydrazide (4.8 g, 15.6 mmol) in CH_3CN (110 mL). The reaction mixture was heated at 75 °C for 4 h, and then concentrated to remove the CH_3CN. The residue was partitioned between ½ saturated brine and EtOAc, the layers were separated and the aqueous layer was further extracted with EtOAc. The combined organics were washed with brine, dried over Na_2SO_4 and concentrated. Purification by ISCO chromatography using 0–10% MeOH/CH_2Cl_2 afforded the oxadiazole (1.61 g, 36%). Step 4: A solution of the oxadiazole (750 mg, 2.58 mmol) and methylamine (6.45 mL of a 2 M solution in THF, 12.9 mmol) in NMP (6.5 mL) was heated to 200 °C in a microwave oven for 1 h. The reaction mixture was diluted with EtOAc and washed with ½ saturated brine and brine. Without drying, the organics were concentrated in vacuo and the solid residue was stirred with 5:1 EtOAc/hexane for 1 h. The solids were collected by filtration to provide the 1,2,4-triazole (660 mg, 84%) as a white powder. Step 5: Conducted as described in Ref. 12 to provide **34** as a waxy yellow solid (75 mg, 53%). ^{1}H NMR ($CDCl_3$) δ 7.46 (d, 1H, J = 8 Hz), 7.32 (d, 1H, J = 8 Hz), 7.10 (t, 1H, J = 8 Hz), 6.95 (t, 1 H, J = 8 Hz), 6.90 (m, 1H), 6.73 (m, 1H), 6.42 (d, 1H, J = 8 Hz), 4.95 (d, 1H, J = 16 Hz), 4.76 (d, 1H, J = 8 Hz), 4.52 (d, 1H, J = 20 Hz), 3.44 (s, 3H), 3.15 (br m, 1H), 2.26 (s, 3H), 1.08 (d, 3H, J = 8 Hz), 1.07 (d, 3H, J = 8 Hz). LCMS (ES) m/z 427.8 $(M+H)^+$.
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