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Bioorganic & Medicinal Chemistry Letters 15 (2005) 3203–3206
Bioorganic & Medicinal Chemistry Letters

Discovery of non-steroidal mifepristone mimetics: Pyrazoline-based PR antagonists

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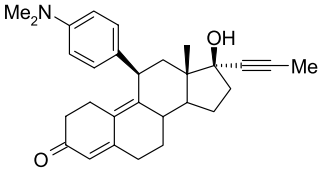
Received 28 March 2005; revised 28 April 2005; accepted 3 May 2005
Available online 31 May 2005

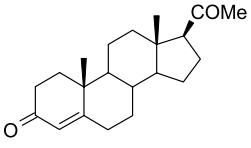
Abstract—Mifepristone is a non-selective antagonist of 3-oxosteroid receptors with both abortifacient and anti-endometriotic activities. Non-steroidal mimetics of mifepristone and progesterone are important templates for modulation of the progesterone receptor (PR). For our PR program, we sought an unexplored, synthetically accessible non-steroidal mimetic of mifepristone, suitable for parallel synthesis of analogues. Docking of compounds into a PR homology model identified 4-substituted pyrazolines, which, when synthesized and tested, exhibited functional antagonism of PR.

Progesterone receptor (PR) antagonists have been targets of medicinal chemistry programs for decades.[1] Mifepristone (RU-486), a clinically approved PR antagonist, effectively terminates pregnancy and offers therapeutic promise for endometriosis, uterine fibroids, and breast cancer.[2] PR, a member of the nuclear receptor superfamily of ligand-dependent transcription factors, undergoes a conformational change upon ligand binding to initiate a cascade of regulatory events with its target genes.[3] Steroidal PR agonists, in combination with estrogens, have been widely used in oral contraception and hormone replacement therapy to oppose estrogen-mediated endometrial cancer risk, yet present potential breast cancer risk with long-term dosing.[4] Thus, there is a need for selective PR modulators to oppose uterine proliferative events while improving safety indices. Non-steroidal PR antagonists/modulators have been identified with desirable reproductive tissue safety profiles in animal models.[5,6]

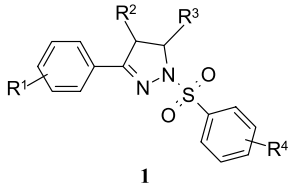
Mifepristone was docked into a PR antagonist homology model generated from the crystal structure of PR complexed to the endogenous agonist progesterone[7] and ERα complexed to the antagonist tamoxifen[8] using the program MVP.[9] The results suggested that the *N*,*N*-dimethylaniline moiety of this compound was responsible for driving the receptor into an antagonist conformation through displacement of the AF2 helix.



Mifepristone



Progesterone



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doi:10.1016/j.bmcl.2005.05.001

In our medicinal chemistry program, we aimed to introduce a variety of substituents to a suitable core, using solution-phase parallel synthesis techniques, to mimic the conformational changes induced by mifepristone. The approach resulted in the design of diarylpyrazolines, a previously unexplored PR chemotype. Related pyridazine-based PR agonists have been disclosed without description of their possible binding mode.[10,11] Synthetic, array-based approaches to the diarylpyrazolines were developed to provide rapid access to both 4- and 5-substituted targets that probe the PR antagonist pocket (e.g., **1**).

Docking of these pyrazoline target molecules into PR co-crystal structures (with agonists progesterone,[6] norethindrone,[12] and mometasone furoate[12]) and the mifepristone homology model suggested that substituted pyrazolines might yield antagonist activity through displacement of the AF2 helix. 4- and 5-Aryl-pyrazolines (R^2 and R^3 of **1**, respectively) were initially proposed. Based on our in silico results, the 3-aryl ring of the pyrazoline overlays suitably with the A-ring of mifepristone, the pyrazoline ring acts as a C-ring moiety, and the benzenesulfonamide portion extends into a 17α-pocket previously observed in the crystal structure of PR bound with mometasone furoate[12] (Fig. 1). The *R*-configured R^2 group occupies a position in the receptor binding pocket very similar to that observed with the *N*,*N*-dimethylaniline group of mifepristone (Fig. 1). Based on SAR reported for the related pyridazine series,[10,11] prototype 4-chlorophenylsulfonamide pyrazoline analogues were synthesized bearing the 3,4-dichlorophenyl substituent to mimic the A-ring of progesterone.

The cyclocondensation of α,β-unsaturated aryl ketones with hydrazine to afford pyrazolines is well known.[13] The key cyclization step in the synthesis of our PR-targeted pyrazolines required an α-substituted-α,β-unsaturated aryl ketone (e.g., **5**) to furnish variably substituted tricyclic intermediate **6** (Eq. 1). Synthetic details are presented below.

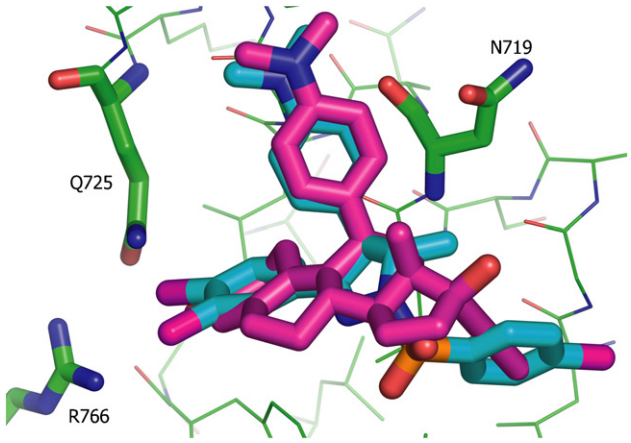
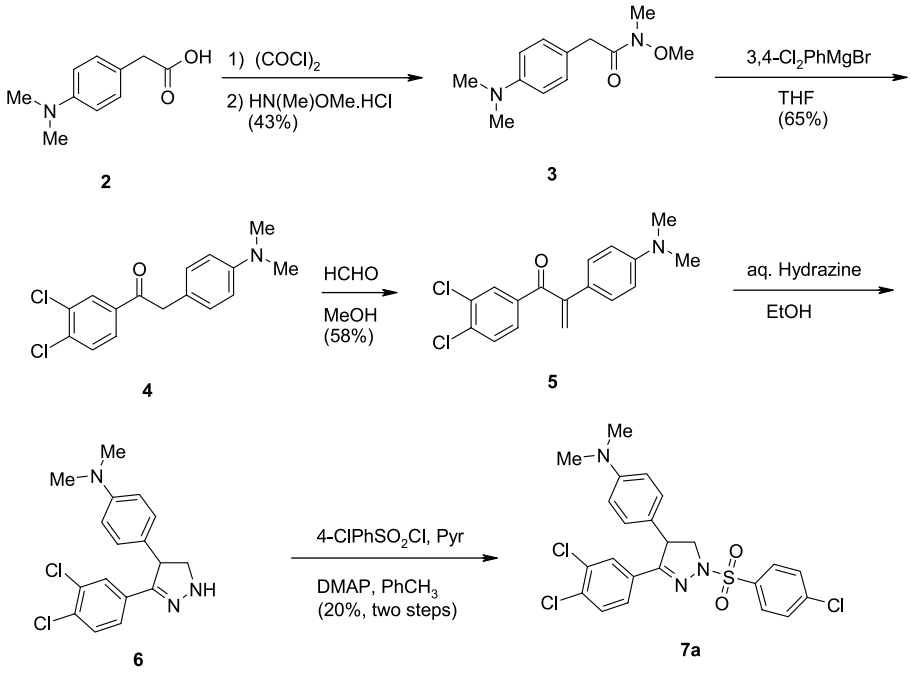


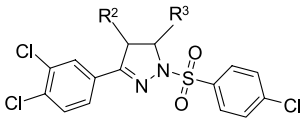
Figure 1. Progesterone receptor ligand binding domain overlay of mifepristone (magenta) with pyrazoline **7b** (cyan) docked into the PR antagonist homology model.

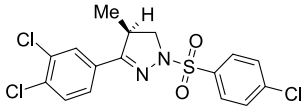
Racemic pyrazoline target **7a** was prepared as follows (Eq. 1).[14] Acid **2** was converted to its corresponding acid chloride with oxalyl chloride followed by Weinreb amide preparation to furnish **3**. The amide **3** was converted to the benzyl ketone **4** by treatment with 3,4-dichlorophenylmagnesium bromide in THF. Condensation of **4** with aqueous formaldehyde in methanol afforded the α,β-unsaturated cyclization substrate **5**. The desired pyrazoline **7a** was then obtained by cyclocondensation of **5** with aqueous hydrazine, followed by in situ sulfonylation of the crude pyrazoline product



(1)

Table 1. PR binding and functional profile of pyrazolines





9b

Compound	R^2	R^3	PR binding[a] pK_i	CV-1 cell[b] pIC_{50}
7a	(*R*/*S*)-4-Me_2NPh	H	7.4	6.1 (95%)
7b	*R*-4-Me_2NPh	H	7.0	5.8 (96%)
7c	*S*-4-Me_2NPh	H	<5.0	<5.5
8	H	(*R*/*S*)-Me	7.4	6.7 (72%)
9a	(*R*/*S*)-Me	H	7.4	NT
9b	*R*-Me	H	7.7	7.6 (51%)
9c	*S*-Me	H	5.7	5.3 (48%)
10	H	(*R*/*S*)-Ph	5.1	6.4 (75%)
11	(*R*/*S*)-Ph	H	7.0	NT
RU-486			8.0	9.6 (100%)

[a] Assay measures compound interaction with the ligand binding domain of PR by displacement of a proprietary fluorescent ligand ($n = 2$, SD = 0.25).[17]

[b] Assay measures inhibition of progesterone-stimulated (4 nM) transactivation of BacMam expressed human PR-B in CV-1 cells using an MMTV-Luc reporter ($n = 2$, SD = 0.20). Mifepristone antagonist efficacy = 100%.[18]

6 with 4-chlorophenylsulfonyl chloride. Select racemic products were purified on a chiral column to isolate the respective enantiomers (Table 1).[15] The absolute configurations of enantiomers **9b** and **9c** were determined by vibrational circular dichroism.[16]

Pyrazoline sulfonamides **7a**–**11** are representatives of arrays of 4- and 5-substituted targets that were synthesized and tested for receptor binding as well as functional activity in CV-1 cells (Table 1). Racemic 4-substituted analogues **7a**, **9a**, and **11** (R^2 = 4-Me_2NPh, Me, and Ph) exhibited strong affinity for PR (pK_i = 7.0–7.4). Furthermore, enantiomerically enriched, *R*-R^2 configured products bound PR with high affinity in agreement with the proposed overlay with the *N*,*N*-dimethylaniline of the steroid RU-486; *S*-R^2-substituted products (**7c**, **9c**) showed low PR binding affinity. Since PR affinity of racemate **9a** resides predominantly in the assigned *R*-enantiomer **9b**, by analogy, we proposed that the enantiomer **7b** also bears the *N*,*N*-dimethylaniline group in the *R*-configuration (Table 1). Also as predicted by homology modeling, these compounds profiled as antagonists, inhibiting progesterone-stimulated PR activity in cells consistent with their binding affinities (pIC_{50} = 5.8–7.6). 5-substituted analogues **8** and **10** (R^3 = Me, Ph) exhibited partial antagonism of PR reporter activity (72–75% efficacy of RU-486). High affinity pyrazolines **7b** and **9b** exhibited >10-fold steroid receptor selectivity over the androgen and glucocorticoid receptors (data not shown).

In summary, we have described the discovery of novel pyrazolines as progesterone receptor ligands. Mifepristone mimetic **7b** exhibits high affinity for the progesterone receptor and a functional profile comparable to those of the steroidal PR antagonist mifepristone. The parallel synthesis of substituted pyrazolines represents a general methodology for the discovery of PR antagonist analogues. 4-Aryl-pyrazolines were proposed to mimic the antagonistic interaction of mifepristone's *N*,*N*-dimethylaniline in the PR ligand binding pocket. This hypothesis was later confirmed through solution of a PR co-crystal structure bound with a representative pyrazoline (data not shown).[19] X-ray crystallographic analysis and homology modeling of these new agents indicate that interaction of the AF2 helix with *R*-configured appendages at four positions of the pyrazoline core can modulate functional PR antagonist efficacy.

Acknowledgments

We thank Karl Erhard for assistance in chromatographic separation of enantiomers and Douglas Minick for determinations of absolute configuration.

References and notes

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14. Compound **7a** was synthesized from **2** as follows: 4-*N*,*N*-dimethylaminophenylacetic acid (**2**, 3.0 g, 16.8 mmol) was dissolved in DCM (100 ml). Oxalyl chloride (3.2 g, 25.1 mmol) and two drops of DMF were added and the reaction was stirred for 1 h at rt. The solvent was evaporated and the residue was re-dissolved in DCM (20 ml) and evaporated. *N*,*O*-dimethylhydroxylamine hydrochloride (1.79 g, 18.4 mmol) was suspended in DCM (50 ml) with addition of pyridine (7 ml). The acid chloride was dissolved in DCM (50 ml) and added dropwise to the amine solution at 0 °C. The resulting

mixture was warmed to rt and stirred for 2 h. The solvent was then evaporated and the residue was re-dissolved in EtOAc. The organic solution was washed with 1 N HCl (aq), satd $NaHCO_3$ (aq), and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by chromatography on silica gel eluted with EtOAc/hexane (40/60) to give **3**. To a solution of **3** (1.6 g, 7.2 mmol) in THF (50 ml) was added 3,4-dichlorophenylmagnesium bromide (0.5 M in THF, 17.3 ml, 8.6 mmol) dropwise over 30 min at 0 °C. The resulting mixture was stirred for 1 h, poured into 1 N HCl (aq), and extracted with three portions of EtOAc. The combined organic layers were washed with satd $NaHCO_3$ (aq) and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by chromatography on silica gel eluted with EtOAc/hexane (7/93) to give **4**. To a solution of **4** (0.30 g, 4.5 mmol) in MeOH (15 ml) were added formaldehyde (37% aq. solution, 4 ml) and piperidine (1 ml). The solution was heated at reflux for 1 h. The solvent was evaporated and the residue was re-dissolved in a small amount of EtOAc and purified by chromatography on silica gel eluted with EtOAc/hexane (5/95) to give **5**. To a solution of **5** (180 mg, 0.56 mmol) in EtOH (25 ml) was added hydrazine hydrate (0.113 g, 2.25 mmol). The reaction was heated at reflux for 3 h. After cooling to rt, the solvent was evaporated and the crude product **6** was re-dissolved in DCM (25 ml). Pyridine (0.09 g, 1.12 mmol), catalytic DMAP, and 4-chlorobenzenesulfonyl chloride (0.24 g, 1.12 mmol) were added, and the resulting solution was stirred at rt for 2 h. The solvent was then evaporated and the residue was dissolved in EtOAc. The organic solution was washed with 1 N HCl (aq), satd $NaHCO_3$ (aq), and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by chromatography on silica gel eluted with EtOAc/hexane (15/85) to give pyrazoline **7a**.

15. Enantiomerically enriched products were isolated from racemates using a 10-μm Chiralpak AD stationary phase (21 × 250 mm) and 10% MeOH/water mobile phase at a flow rate of 50 ml/min with detection at 254 nm.
16. The absolute configurations of **9b** (assigned as *R*-enantiomer) and **9c** (assigned as *S*-enantiomer) were determined on a Bomem Chiral-IR Vibrational Circular Dichroism spectrometer operated at 4 cm^{-1} resolution in carbon tetrachloride at a concentration of 20 mg/ml. For a reference, see: Devlin, F. J.; Stephens, P. J.; Osterle, C.; Wiberg, K. B.; Cheeseman, J. R.; Frisch, M. J. *J. Org. Chem.* **2002**, *67*, 8090, and references cited therein.
17. The PR fluorescence polarization binding assay measures compound interaction with the ligand binding domain of PR (590 nm) by displacement of a fluorescent steroidal progesterone mimetic (Panvera catalog number P2964; K_d = 10 nM). The response is expressed as a pK_i.
18. The functional assay measures compound-mediated interaction of the PR-B isoform with the MMTV luciferase reporter to calculate compound potency and efficacy in BacMam transduced, progesterone-stimulated (4 nM) CV-1 cells. The antagonist response is expressed as a pIC_{50} (RU-486 pIC_{50} = 9.8, 100% efficacy). Comparable potencies and efficacies were observed for a limited set of compounds in a T47D cell alkaline phosphatase assay.
19. Details will be reported in a forthcoming full paper.