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Array synthesis of progesterone receptor antagonists: 3-Aryl-1,2-diazepines

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Abstract—New non-steroidal chemotypes are required for the development of drugs targeting the steroid hormone receptors. The parallel array synthesis of 3-aryl-1,2-diazepines employing solid-supported reagents is described. The resulting compounds demonstrated high affinity binding to the progesterone receptor.

Progesterone receptor (PR) ligands have utility as treatments for a range of reproductive diseases and have been the target of several medicinal chemistry programs.[1] The PR antagonist mifepristone (RU-486) is prescribed for termination of pregnancy and also offers promise for the treatment of endometriosis, uterine fibroids, and breast cancer.[2] Recently, non-steroidal PR antagonists and partial agonists have been identified with improved reproductive tissue safety profiles.[3,4] Nevertheless, new chemotypes that are amenable to high-throughput synthesis are useful to further structural understanding of PR modulation.

3-Aryl-pyridazine PR agonist **1**[5] and 3-aryl-pyrazoline PR antagonist **2**[6] have been previously disclosed. The binding mode of the pyrazolines has been reported, wherein the 3-aryl moiety occupies the steroid A-ring binding pocket of PR.[6] In our medicinal chemistry program, we aimed to introduce a variety of pharmacophores to a structurally related 1,3-disubstituted-1,2-diazepine core via parallel synthesis. The strategy furnished *N*-arylsulfonyl-3-aryl-1,2-diazepine based PR ligands. The synthesis of high affinity 3-aryl-1,2-diazepines **3** is disclosed herein.

The targeted synthesis of the 3-aryl-1,2-diazepine core was first reported by Koenig and Wermuth.[7] Prior to this disclosure, 1-*N*-alkyl-3-aryl-1,2-diazepines had been isolated as pyrolysis side products.[8] The template has also been employed to prepare a diazepine thiosemicarbazide.[9] The key annulation step in the synthesis of diazepine targets involved treatment of phenyl-δ-chlorobutyl ketones with hydrazine (Scheme 1). Unfortunately, decomposition of the product occurred upon standard aqueous workup. Modifications to the protocol were required to allow parallel synthesis of the diazepine targets. Details of the improved synthesis are presented below.

Compounds were prepared starting from substituted aryl-δ-chlorobutyl ketones **4** (Scheme 1, for monomers **4**, see Ref. 14).[10] Initial experiments showed that ring closure using 4 equiv of hydrazine hydrate in ethanol at reflux overnight gave good yields of the diazepine intermediates **5**. Solvation with 2-propanol gave improved annulation yields when *o*-substituted aryl ketones (R^1) were employed. The workup consisted of a rapid filtration of the reaction to remove the majority of the insoluble hydrazine hydrochloride byproduct, followed by concentration of the filtrate to yield the crude product. The remaining traces of hydrazine hydrochloride were removed by suspending the residue in dry dichloromethane, followed by filtration and concentration of the solution. Attempts to chromatograph or extract the diazepine intermediate resulted in typically >50% decomposition of product. In contrast, the intermediates were stable at room temperature for weeks if kept dry. The diazepine intermediate **5** could also be precipitated from a dry diethyl ether solution

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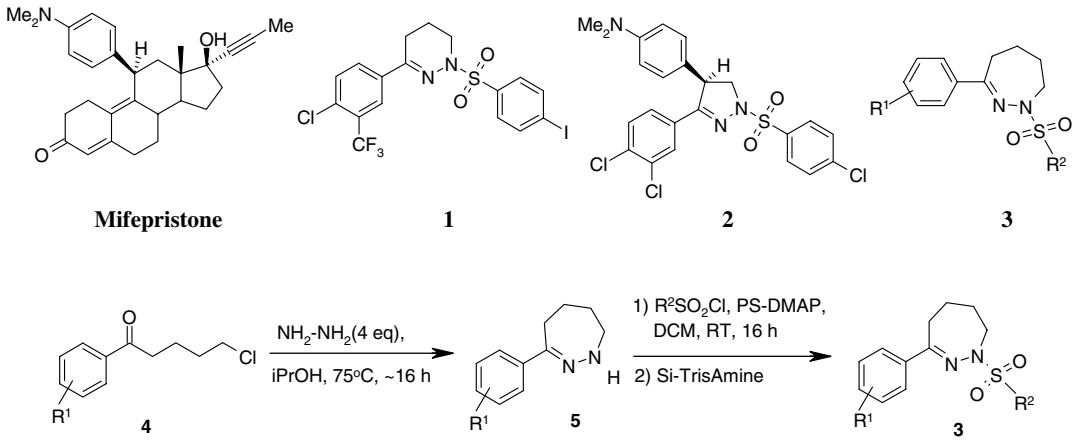
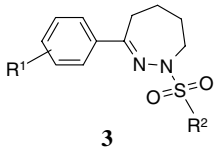


Scheme 1. Parallel synthesis of diazepines.

by addition of HCl in ether to give a stable, hygroscopic HCl salt. Cyclization could also be effected using microwave heating at temperatures up to 170 °C for 5–30 min; the forcing conditions were required to form some of the diazepines with *o*-substituents (e.g., R^1 = 2-bromo, 2-trifluoromethyl). In some of these sterically hindered cases, additional hydrazine hydrate was required, but 4 equiv was adequate for most reactions. Use of fewer than 4 equiv of hydrazine led to side products derived from incomplete ring closure and polymerization. Using this methodology, solution-phase parallel synthesis in a 96-well format allowed the isolation of >1300 sulfonamide products **3** without chromatography on either step.

Standard conditions for the preparation of sulfonamides **3** from intermediates **5** (1 equiv arylsulfonyl chloride, pyridine or 4-dimethylaminopyridine in dichloromethane) gave variable results (12–47% isolated yields). Typically, poorer yields were realized as scale was increased (<20%). Presumably, formation of HCl promoted decomposition of the diazepine intermediates **5**. An alternate route employed the 'catch and release' method wherein 4 equiv of arylsulfonyl chloride was reacted with 4 equiv of polystyrene-supported 4-dimethylaminopyridine (PS-DMAP) for 2 h.[10] The resin was washed with dichloromethane to remove the excess arylsulfonyl chloride followed by addition of diazepine in dichloromethane to give products of >90% purity after stirring overnight. The formation of traces of decomposition products could be avoided through addition of merely a catalytic amount of diisopropylethylamine (DIPEA) or triethylamine to the resin activation medium to buffer the reaction mixture. Finally, Silicycle Si-TrisAmine® scavenger was added and agitation continued for 1–2 more hours to remove any remaining arylsulfonyl chloride. Filtration and evaporation of solvent furnished diazepines **3** with purities acceptable for biological testing (85–100%, Table 1).

Table 1. Isolated chemical yields and PR activities of diazepines



3

Compound	R^1	R^2	Isolated yield (%)	LC purity (%)	PR Bdg.[a] pK_i	CV-1 cell[b] pIC_{50} (% max)
3a	3,4-Cl_2	5-Br-2-thienyl	64	85	7.6	6.5 (60)
3b	3,4-Cl_2	5-Cl-2-thienyl	93	100	7.4	NT[c]
3c	3,4-Cl_2	2,4-Cl_2-Ph	32	94	7.3	6.7 (82)
3d	4-CN	2-F–Ph	86	100	6.0	6.4 (89)
3e	4-CN	5-Br-2-thienyl	85	100	7.2	NT
3f	4-Cl	3-Cl–Ph	100	100	6.2	5.9 (89)
3g	2-Cl	5-Cl-2-thienyl	72	96	6.7	6.2 (68)
3h	3-F	4-Cl–Ph	82	95	6.1	6.3 (91)
3i	3-Cl	3-Cl–2-Me–Ph	100	96	6.5	5.7 (86)
3j	3-Br	5-Br-2-thienyl	87	93	7.2	6.6 (71)
3k	4-NO_2	4-CF_3O–Ph	74	92	7.3	6.8 (65)
RU-486					8.0	9.6 (100)

[a] Assay measures compound interaction with the ligand binding domain of PR by displacement of a fluorescent ligand ($n \geqslant 3$, SD = 0.25).[11]

[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 ($n \geqslant 2$, SD = 0.20).[12]

[c] NT, not tested.

Sulfonamides **3a**–**k** were highly active representatives of the >1300 1,3-disubstituted diazepines that were tested for progesterone receptor binding affinity[13] and functional antagonism in CV-1 cells (Table 1, array compounds with highest affinity R^1 groups shown). Products wherein the R^1 group was 3,4-dichloro exhibited high affinity for PR (e.g., **3a**–**c**). The 5-bromo-2-thiophene-sulfonamide **3a** ($pK_i = 7.6$) bound to PR with similar affinity to the steroidal antagonist RU-486 ($pK_i = 8.0$). Dichloro substitution (e.g., **3a**,**b**) on the 3-aryl moiety provided higher affinity ligands than those with monochloro substitution (e.g., **3f**,**g**). Array members wherein R^1 was H, 3-methyl, 4-methyl, or 4-methoxy did not display measurable PR binding ($pK_i < 5.0$). Halogenated thiophenes represented one of the optimal arene groups at R^2. Generally, halo-aryl substituents at R^2 were necessary for high affinity PR binding. Electron-rich and bicyclic aromatic R^2 groups gave poor PR binding. Consistent with the related pyrazoline series (and unlike the pyridazine based, breast cell stimulatory agonist series[5]) the diazepines generally antagonized progesterone-stimulated reporter activity in CV-1 cells (for **3c**, $pIC_{50} = 6.7$).[6] Less than 5% of array members exhibited PR agonist activity. While a small percentage of array members bound to the glucocorticoid receptor with ca. micromolar affinity ($pK_i = 6.0$–6.3), the diazepines **3** generally exhibited >50-fold selectivity for PR over the other steroid hormone receptors (data not shown).

In summary, we have described a practical, two-step array synthesis of new progesterone receptor binding 3-aryl-1,2-diazepines. Solution-phase parallel synthesis using solid-supported reagents and resin scavengers facilitated the synthesis of >1300 products while removing the need for purification of the intermediates and products by column chromatography. As an application of this chemistry, key intermediates **4a**–**k** were carried forward to biologically active PR ligands **3a**–**k**.

Acknowledgments

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10. Diazepine product **3a** was prepared as follows: 3,4-dichlorophenyl-δ-chlorobutyl ketone[14] (**4a**, 2.8 g, 10.5 mmol) was dissolved in 2-propanol (90 mL) in a 120 mL bottle and then hydrazine hydrate (2.0 mL, 41.9 mmol) was added. The bottle was heated in a stirring heat block at 75 °C overnight (~16 h) and allowed to slowly cool to rt. The colorless precipitate was removed by filtration into a large test tube and all of the solvent and excess hydrazine were removed in vacuo (using a Savant Speedvac overnight). The remaining slurry was dissolved in anhydrous dichloromethane (DCM) and filtered to remove the hydrazine HCl salt. The filtrate was concentrated in vacuo to give **5a** as an oil (2.5 g, 99% crude yield, ~95% purity by both LC/MS and ^{1}H NMR). Second step: to PS-DMAP resin (168 mg, 1.5 mmol/g, dried under high vacuum at 50 °C overnight) in one well of a 96-well Scigene Flexchem reactor was added a solution of DIPEA (0.02 mL) and anhydrous DCM (1 mL) at rt. This mixture was treated with 5-bromo-2-thiophenesulfonyl chloride (0.66 mL, 0.75 M in anhydrous DCE) via a Gilson-215 liquid handler and the plate sealed. The plate was rotated and agitated for 2 h, and the reactor was placed into the wash manifold under vacuum to remove the liquid from the resin. The resin was further washed with 0.5 mL anhydrous DCM (per well) and the bottom cover was reinstalled on the reactor. Compound **5a** (24 mg, 0.10 mmol in 1 mL anhydrous DCM) was added to the activated resin. The plate was resealed and rotated at rt for 16 h. The top cover was removed and Silicycle Si-Trisamine® scavenger resin (135 mg) was added to each well and the plate resealed and agitated for 2 h. The reaction well contents were filtered into a 96-well Multichem plate and washed with DCM (3× 0.5 mL). The filtrate was concentrated in vacuo to afford **3a** as a solid (30 mg, 64% yield, 85% purity by LC/MS).
11. The PR fluorescence polarization binding assay[6,15] 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.
12. The functional assay[6] 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} and the percentage of maximal efficacy is measured relative to the fully efficacious antagonist RU-486 (RU-486 $pIC_{50} = 9.6$, 100% efficacy).
13. Approximately 289 of the >1300 array members were progressed from single concentration testing to the full-curve PR binding assay. Eighteen members exhibited pK_i 7–7.6, 119 members at pK_i 6.0–6.9, 127 members at pK_i 5.0–5.9, and 25 members at pK_i <5.0.
14. δ-Chlorobutyl aryl ketones were purchased from Rieke metals. The following monomers were purchased: R^1 = 3- or 4-substituted F, Cl, Br, Me, CF_3, and NO_2; R^1 = H, 4-OMe, 4-CN, 3,4-F_2, 3,4-Cl_2, and 3-Me-4-Br.
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