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1*H*-Pyrazolo-[3,4-*c*]cyclophepta[1,2-*c*]thiophenes: A Unique Structural Class of Dopamine D_4 Selective Ligands

Andrew Thurkauf,[a] Xi Chen,[a] Suoming Zhang,[a] Yang Gao,[a] Andrzej Kieltyka,[a] Jan W. F. Wasley,[a] Robbin Brodbeck,[a] William Greenlee,[b] Ashit Ganguly[b] and He Zhao[a,*]

[a]*Neurogen Corporation, 35 Northeast Industrial Road, Branford, CT 06405, USA*
[b]*Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA*

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Abstract—A series of novel 1*H*-pyrazolo-[3,4-*c*]cyclophepta[1,2-*c*]thiophenes was prepared and screened at selected dopamine receptor subtypes. Compound **4** (NGB 4420) displayed high affinity and selectivity (>100-fold) for the D_4 over D_2 and other CNS receptors. This compound was identified as a D_4 antagonist via its attenuation of dopamine agonist-induced GTPγ^{35}S binding at D_4 receptor.

Most classical neuroleptics are generally believed to exert their antipychotic actions through interaction with dopamine receptors and the antipsychotic efficacy of these agents has been shown to have a strong correlation with their affinity for the dopamine D_2 receptor subtype. The neuroleptic agent clozapine has been referred to as an atypical antipsychotic agent because it does not induce the extrapyramidal motor side effects characteristic as many other 'typical' antipsychotic medications such as haloperidol and chlorpromazine. A large body of research has been carried out in an effort to identify the properties of clozapine which leave it devoid of this liability. The broad spectrum of CNS receptors for which clozapine has significant affinity has made this a daunting task.[1]

In 1991, Seeman characterized a new dopamine receptor subtype. This new receptor was found to be related to the D_2 receptor in that stimulation of the receptor by dopamine leads to an inhibition of c-AMP production by adenylate cyclase.[2] The localization of this new receptor subtype, termed D_4, in the limbic areas of the central nervous system, coupled with the upregulation of this receptor found in postmortem autoradiography study of the schizophrenic brain, led to an intense effort in the following years to identify specific D_4 antagonists.[3] The observation that clozapine, unlike typical neuroleptics, displayed a selectivity for the D_4 receptor over the D_2 sparked the theory that a selective D_4 antagonist might share its atypical neuroleptic profile.

The medicinal chemistry efforts in the D_4 area lead to the identification of a wide array of chemical entities with selectivity for this receptor subtype over that of D_2.[4–19] Although the search for D_4 selective agents has been fruitful, interest in this area was dampened by the results of a number of clinical trials, notably those of L-745,870, CP-293,019 and U-101,387 (Fig. 1), which failed to show significant efficacy against either positive or negative symptoms of schizophrenia.[20–22] These trials bring into question the relevance of this receptor subtype in the etiology of psychosis.

As part of our efforts within the area, screening of a Schering-Plough compound library for D_4 activity led to the identification of SCH 26682 (**1**) (Fig. 1) as a compound with significant D_4 activity. Although, in retrospect, identification of a new D_4 selective entity seems unremarkable, SCH 26682 stands out from other known D_4 selective compounds in a number of ways. In terms of basicity, the presence of piperazine, piperidine or pyrrolidine subunits lead that many D_4 selective compounds show the pK_a values around 7.[23,24] In contrast, the weakly basic pyrazole moiety of SCH 26682

*Corresponding author. Tel.: +1-203-488-8201x3077; fax: +1-203-483-7027; e-mail: hzhao@nrgn.com

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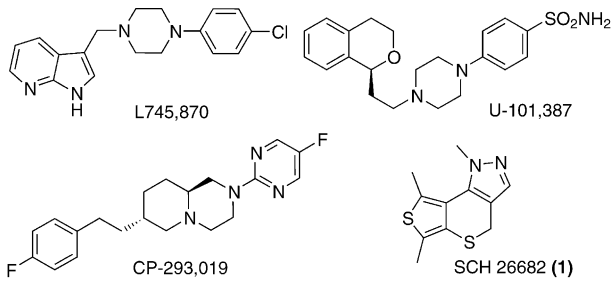
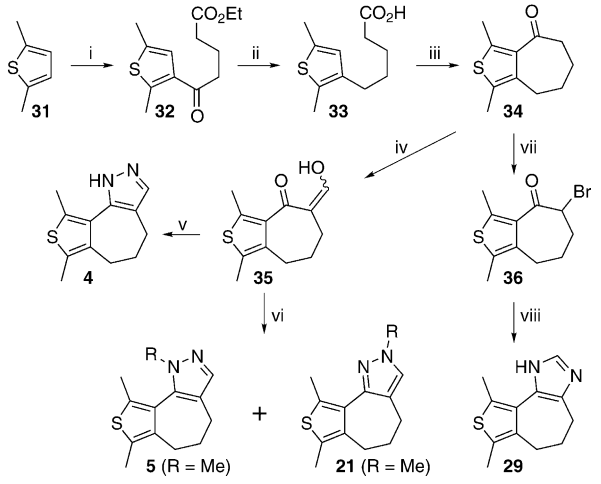


Figure 1.

imparts the pK_a of only 3.06. In addition, SCH 26682 has a unique fused thiophene structure. Unfortunately, a potentially poor pharmacokinetic profile for SCH 26682 was indicated by a $T_{1/2}$ of less than 1 min upon exposure to human liver microsomes. In our examination of SCH 26682, we considered that replacement of the sulfur in the thioether might prove beneficial to the pharmacokinetic profile of the resulting derivatives. In this paper, we disclose the details of structure–activity relationship (SAR) analysis of this unique structural class of D_4 selective agents.

The synthetic route towards many of the described pyrazolocycloheptathiophenes (**1**–**10**, **20**, and **21**) is illustrated by the representative preparation depicted in Scheme 1. The chlorinated (**14**–**19**), furanyl (**11** and **12**) and phenyl (**22**–**24** and **28**) analogues were prepared in similar fashion by use of appropriate starting materials in the first step. The Friedel–Crafts acylation of 2,5-dimethylthiophene **31** with ethyl glutaryl chloride produced ketoester **32**. Reduction of the ketone carbonyl using hydrazine and potassium hydroxide in triethylene glycol at 190 °C yielded the 5-(2,5-dimethylthiophen-3-yl)valeric acid **33** in 58% overall yield from **31**. Cyclization using polyphosphoric acid at 140 °C afforded cyclohepta[1,2-*c*]thiophene **34** in 80% yield. Hydroxymethylene ketone **35** was prepared in quantitative yield



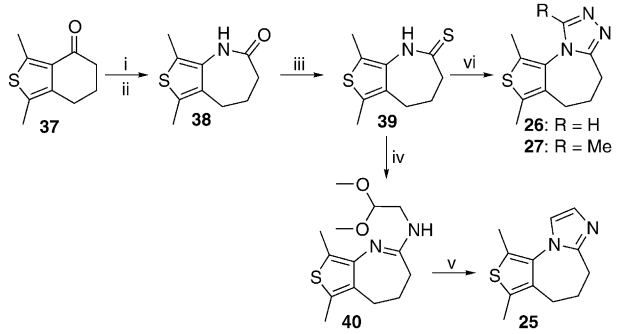
Scheme 1. Reagents and conditions: (i) $EtO_2C(CH_2)_3COCl$, $AlCl_3$, CH_2Cl_2, −10 °C, 30 min, then 0 °C, 1 h; (ii) NH_2NH_2, KOH, triethylene glycol, 140 °C, 10 min, then distillation of solvent and NH_2NH_2 at 180 °C, then 205 °C, 16 h, 58% in two steps; (iii) PPA, 140 °C, 2 h, 80%; (iv) Na, EtOCHO, PhMe, 60 °C, 16 h, 100%; (v) $NH_2NH_2{\cdot}H_2O$, MeOH, rt, 1 h, 44%; (vi) $RNHNH_2$, MeOH, rt, 1 h, >50%; (vii) Br_2, HOAc, 70 °C, 0.5 h, 100%; (viii) $HCONH_2$, 130 °C, 7 h, 23%.

through the condensation of the anion of **34** with ethyl formate in toluene. The resulting aryl fused ketoaldehyde structure would serve as the common intermediate in the preparation of both 1- and 2-substituted pyrazoles through condensation with the appropriate hydrazine. For example, reaction of **35** with 1.3 equiv of methylhydrazine in methanol provided compounds **5** and **21** in a ratio of 6:4 with an overall yield of 92%. With sterically larger hydrazines, the isomer ratio was more dramatically skewed toward the 1-substituted pyrazoles.

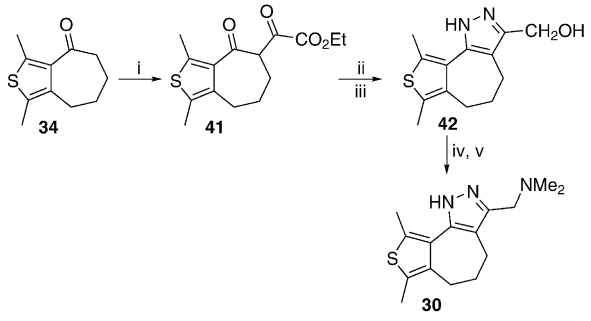
The imidazole **29** was prepared via the alpha bromination of **34** in acetic acid followed by heating in formamide at 130 °C as illustrated in Scheme 1.

Imidazole **25** and triazoles **26** and **27** were prepared from lactam **38**, the Beckmann rearrangement product of cyclohexanone **37** (Scheme 2). Treatment of **38** with P_2S_5 in dioxane gave thiolactam **39**. Reaction of **39** with formic or acetic hydrazide at 150 °C provided triazole **26** or **27**, respectively. Reaction of **39** with aminoacetaldehyde dimethyl acetal followed by cyclization in concentrated sulfuric acid gave imidazole **25**.

Aminomethylpyrazole **30** was prepared in five steps from ketone **34** (Scheme 3). Condensation of the anion of **34** with diethyloxalate followed by treatment of the intermediate ketooxalate **41** with hydrazine and reduction gave the hydroxymethylpyrazole **42**. Treatment of



Scheme 2. Reagents and conditions: (i) $NH_2OH{\cdot}HCl$, $EtOH/CH_2Cl_2$, rt, 16 h; (ii) PPA, $EtOH/CHCl_3$, rt, 16 h, 58% in two steps; (iii) P_2S_5, dioxane, 130 °C, 1 h, 82%; (iv) $H_2NCH_2CH(OMe_2)_2$, ^{i}PrOH, reflux, 18 h, 82%; (v) concd H_2SO_4, rt, 3 h, 75%; (vi) $RCONHNH_2$, MeOH, 140 °C, 1 h, >80%.



Scheme 3. Reagents and conditions: (i) $(CO_2Et)_2$, NaH, THF, rt, 24 h; (ii) NH_2NH_2, MeOH, rt, 4 h; (iii) LAH, THF, rt, 2 h, 42% in three steps; (iv) $SOCl_2$, CH_2Cl_2, rt, 3 h; (v) aqueous Me_2NH, rt, 24 h, 82% in two steps.

42 with thionyl chloride and reaction of the chloromethyl intermediate with *N*,*N*-dimethylamine provided the desired product **30**.

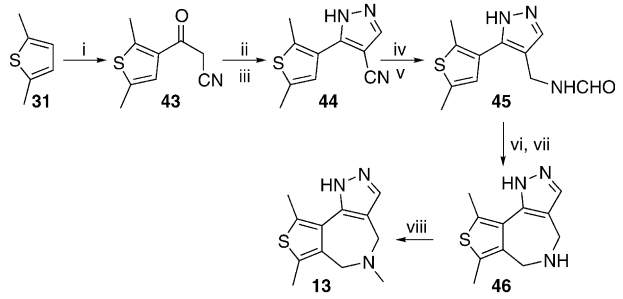
Finally, cycloheptenimine **13** was prepared in eight steps as depicted in Scheme 4. In this route, 2,5-dimethylthiophene **31** was condensed with 2-cyanoacetyl chloride in the presence of aluminum chloride to give **43**. Hydroxymethylenation followed by treatment with hydrazine gave the 4-cyanopyrazole **44**. The cyano function was reduced and formylated to provide **45** which was cyclized and reduced to the cycloheptenamine **46**. Finally, reductive methylation of **46** using formic acid and sodium cyanoborohydride afforded the desired tertiary amine **13**.

Affinity at dopamine receptors was determined via standard competitive displacement assays using receptors cloned from human.[25] The binding affinity data are summarized in Table 1.

It can been seen from the binding results in Table 1 that, while replacement of the sulfur in **1** by a methylene (**3**) maintained D_4 activity, the bioisosteric substituent ethylene proved more successful (**5**) in this regard. Within both the cyclohexyl and cycloheptyl series *N*-methylation of the pyrazole at the 2 position as in **3** and **5** improved the overall affinity for the D_4 site over the unsubstituted derivatives **2** and **4** although the D_4/D_2 selectivity was somewhat diminished. Methylation at the *N*-1 position of the pyrazole (**20** and **21**) significantly lowered D_4 affinity. Larger *N*-2 substituents (**6**–**9**) further diminished both D_4 affinity and selectivity.

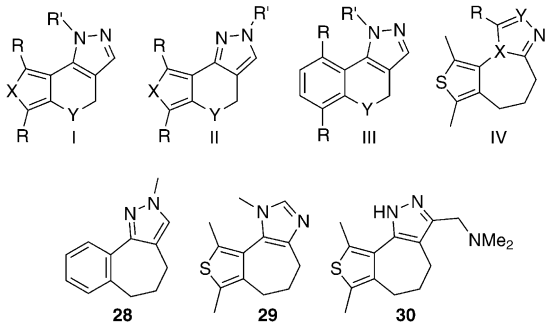
Although the sulfur-to-ethylene transformation was successful in the maintaining receptor profile, it did not lead to an appreciable modification of human microsomal $T_{1/2}$. We postulated benzylic oxidation as a primary metabolic transformation. We felt that *gem*-dimethyl substitution at the single non-benzylic methylene of **4** might block access by hepatic enzymes to both benzylic carbons within the carbocycle (**10**). Replacement of the *ortho* methyl groups of the thiophene by chlorine might also negate oxidation at these positions (**14**) and also lower the oxidation potential of the thiophene sulfur atom, whose oxidation products were previously determined to be inactive at the receptor (data not shown). The *gem*-dimethyl **10** displayed low affinity for D_4. The 2,5-dichlorothiophene **14** displayed lower overall affinity for the receptors than the corresponding 2,5-dimethylthiophene **4** and the SAR of the corresponding 2-alkylated pyrazole derivatives (**15**–**19**) was also in agreement with what seen for **4**, in that increasing size of the *N*-substituent resulted largely in decreasing affinity and selectivity for the D_4 receptor.

Replacement of the thiophene ring by a simple phenyl isostere largely eliminated D_4 activity (**22**, **23**, and **28**) although this loss was less prominent when the di-*ortho*-methyl group of **4** was returned to the corresponding aromatic positions as in compound **24**. The furan derivative **11** was also less active, although 2-methylation (**12**) of this compound returned potent D_4 activity without much D_4/D_2 selectivity.



Scheme 4. Reagents and conditions: (i) $NCCH_2COCl$, $AlCl_3$, CH_2Cl_2, 0 °C, 1 h, 89%; (ii) $Me_2NCH(OMe)_2$, 85 °C, 1 h; (iii) $NH_2NH_2{\cdot}H_2O$, MeOH, rt, 1 h, 43% in two steps; (iv) LAH, THF, rt, 48 h; (v) HCO_2Et, 0.5 N NaOH, rt, 48 h, 83% in two steps; (vi) PPA, 140 °C, 2 h; (vii) $NaBH_4$, EtOH, rt, 2.5 h, 79% in two steps; (viii) 37% aqueous HCHO, $NaBH_3CN$, MeOH, 60 °C, 2 h, 52%.

Table 1. Binding affinities



Compd	Structure	R	R′	X	Y	K_i (nM)	
						D_2	D_4
1	I	Me	Me	S	S	111	3
2	I	Me	H	S	CH_2	4166	170
3	I	Me	Me	S	CH_2	462	26
4	I	Me	H	S	CH_2CH_2	2066	12
5	I	Me	Me	S	CH_2CH_2	176	2
6	I	Me	Et	S	CH_2CH_2	402	22
7	I	Me	*n*-Pr	S	CH_2CH_2	885	97
8	I	Me	*i*-Pr	S	CH_2CH_2	555	138
9	I	Me	Ph	S	CH_2CH_2	>10,000	1578
10	I	Me	H	S	CH_2CMe_2	>10,000	1297
11	I	Me	H	O	CH_2CH_2	ND	275
12	I	Me	Me	O	CH_2CH_2	28	11
13	I	Me	H	S	CH_2NMe	ND	>10,000
14	I	Cl	H	S	CH_2CH_2	534	169
15	I	Cl	Me	S	CH_2CH_2	416	14
16	I	Cl	Et	S	CH_2CH_2	489	168
17	I	Cl	*n*-Pr	S	CH_2CH_2	>10,000	3548
18	I	Cl	*i*-Pr	S	CH_2CH_2	>10,000	1563
19	I	Cl	Bn	S	CH_2CH_2	>10,000	>10,000
20	II	Me	Me	S	CH_2	ND	2866
21	II	Me	Me	S	CH_2CH_2	641	228
22	III	H	H	—	CH_2CH_2	>10,000	>10,000
23	III	H	Me	—	CH_2CH_2	>10,000	>10,000
24	III	Me	H	—	CH_2CH_2	>10,000	425
25	IV	H	—	N	CH	>10,000	301
26	IV	H	—	N	N	>10,000	4325
27	IV	Me	—	N	N	>10,000	462
28	—	—	—	—	—	>10,000	507
29	—	—	—	—	—	>10,000	1293
30	—	—	—	—	—	ND	2563

Table 2. Receptor-binding profile for compound **4** (NGB 4420)

Receptor	D_1	D_2	D_3	D_4	D_5
K_i (nM)	> 10,000	2067	> 10,000	12	> 10,000
Receptor	5-HT_{1a}	5-HT_2	α1	α2	
K_i (nM)	> 1000	2087	> 10,000	> 7800	

The loss of potency by replacement of pyrazole with selected imidazoles and triazoles (**25**–**27** and **29**) indicated that the pyrazole plays a key role as a pharmacophore. Attempts to improve solubility via the amine **30** or insertion of a nitrogen into the cycloheptane ring (**13**) were largely unsuccessful.

As compound **4** (NGB 4420) displayed a 100-fold selectivity for the D_4 over D_2 receptor subtype, it was chosen for further examination of its binding profile against related CNS receptors (Table 2). Compound **4** displayed no appreciable affinity (> 5000 nM) for the D_1, D_3 and D_5 receptor subtypes. When tested against selected cloned human serotonin and norepinephrine receptors only micromolar affinities were observed. Extended screening against a battery of 82 receptors, ion channels and enzymes systems (Panlabs; Bothell, WA, USA) revealed no affinity greater than 2 μM.

The functional activity of **4** was assessed by measuring its ability to block the agonist-induced binding of GTPγ^{35}S. The GTPγ^{35}S binding functional assay was used to demonstrate a dose dependent agonist stimulation by a full agonist.[25] Compound **4** and the reference antagonist haloperidol demonstrated a baseline level of activity when used alone suggesting that they possess no agonist activity at the human $D_{4.2}$ receptor. On the other hand, **4** and haloperidol completely reversed the agonist-stimulated GTPγ ^{35}S binding in a dose-dependent fashion with EC_{50} values of 9 and 17 nM, respectively. The GTPγ^{35}S binding assay data suggest that compound **4** functions as an pure antagonist at the human $D_{4.2}$ receptor.

In conclusion, using the lead compound **1** as reference, a systematic SAR study has been carried out with the goal of identifying compounds as selective dopamine D_4 antagonists. Compound **4** (NGB 4420) displayed a good selectivity for the D_4 over D_2 and other related CNS receptors. Its human microsomal stability ($T_{1/2} = 6$ min) is not ideal, but much better than that of compound **1** ($T_{1/2} < 1$ min). In addition, the unique structure of compound **4** with different physicochemical properties (MW = 218.2; mLogP = 2.48; PSA = 22.31) compared to other D_4 antagonists (Fig. 1) may play a significant role as for further biological evaluation.

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