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Mitigating Heterocycle Metabolism in Drug Discovery

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INTRODUCTION

In the past few decades, drug metabolism research has played an ever increasing role in the design of drugs.[1−3] In vitro metabolism assays[4] have become an integral part of the routine profiling of compounds made in drug discovery.[5] The data from these assays have allowed medicinal chemists to focus their efforts on compounds with improved metabolic stability.[6] Detailed metabolite identification studies are also done more routinely, which provide information on how to strategically replace or block metabolically labile sites.[7] Additionally, in vivo PK studies are regularly conducted in drug discovery, which helps to build in vitro−in vivo PK relationships.[4] The positive influence that these advances in PKDM sciences have had on drug discovery is reflected in the fact that fewer drug candidates fail in the clinic for PKDM related issues.[8] This suggests that medicinal chemists are successfully integrating the data generated by their PKDM colleagues into the design of compounds with fewer metabolic liabilities.

Extensive data from metabolism studies have allowed medicinal chemists to develop general principles for reducing compound metabolism. These methods include, but are not limited to, reducing lipophilicity, altering sterics and electronics, introducing a conformational constraint, and altering the stereochemistry of their compounds. While no single method is able to solve every metabolic problem, these principles do give medicinal chemists guidance on how to improve the metabolic liabilities of their compounds. If the specific site of metabolism is known, medicinal chemists block the site, typically with a fluorine, or replace the metabolically labile group with a bioisostere.[9] While several authors have reviewed these techniques for reducing metabolism,[5,10,11] there is no review that summarizes different approaches to improving the metabolic stability of heterocycles. In this review, we summarize examples where changes were made at or near the heterocycle to improve metabolic stability. By summarizing these examples, we hope to provide a useful guide to medicinal chemists as they attempt to improve the metabolic profile of their own heterocyclic compounds.

The majority of the examples that are included in this review came from searching the online open access database CHEMBL.[12] In addition to having pharmacology data on compounds from the medicinal chemistry literature, CHEMBL has over 120 000 points of data on the ADMET properties of compounds. With the help of the visualization software Spotfire, we were able to cull examples from the CHEMBL ADMET data that focused on heterocycles. We also identified examples from papers that cite leading reviews in the drug metabolism field[13−18] and were present in other recent reviews on drug metabolism.[19−22] The main criteria that we placed on the examples selected for this review was that the change made to improve metabolism had to occur at or near the heterocycle and nowhere else on the molecule. This allowed us to eliminate examples where a change made to a compound away from the heterocycle may have influenced the metabolism.

The data that we included in this review is predominantly from in vitro microsomal stability studies. However, we have included some data from bioactivation studies and in vivo PK studies to provide additional information about the overall metabolic profile. In several instances, the compound with the improved metabolic profile also became the lead compound in the paper, so we felt that including the data on the intended target was informative even though this is not a discussion point for the review. Of course, in some examples when the heterocycle was modified to improve metabolic stability, the activity at the intended biological target diminished. However, we felt that these examples of improved metabolic stability would still be of value to the reader. In the discussion below, we have organized the review by first discussing saturated heterocycles and then heteroaromatic compounds. Within each section we have organized the discussion by ring size.

SATURATED HETEROCYCLES

Saturated heterocycles are most prone to metabolism at the position adjacent to or directly on the heteroatom.[16,23] Therefore, strategies used to reduce metabolism of these rings involve techniques to block the site of metabolism, change the electronics of the ring, or reduce the hydrophobicity of the ring. Since lipophilicity and charge influence metabolism, we have tabulated the cLogP, cLogD$_{7.4}$,[24] and pK_a[25,26] for some of the more common saturated heterocycles (Table 1).

Saturated Four-Membered Rings. Of the saturated heterocycles containing one heteroatom, azetidine and oxetane are among the least lipophilic, so one would expect these rings to be least likely to undergo metabolism when compared to their larger ring counterparts. In fact, in some of the examples show below, the azetidine and oxetane ring analogues were more metabolically stable than analogues containing a larger ring (see Figures 5, 8, 12, and 14). However, there are some reports where the four-membered saturated heterocycle was modified to improve metabolic stability. For example, in their work on histamine receptor 3 (H_3R) inverse agonists for the treatment of obesity, Pierson et al. found that the derivative containing the oxetane **1** was less metabolically stable in rat liver microsomes (RLM) than the cyclobutyl analogue (**2**) (see Figure 1).[27] While the oxetane analogue **1** was less lipophilic than cyclobutyl analogue **2**, the pK_a of the adjacent piperidine ring was >9 for analogue **2**, while the oxetane analogue **1** had a pK_a = 6.4. This pK_a difference may explain why the cyclobutyl analogue **2** is more stable, since at physiological pH, this

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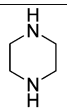
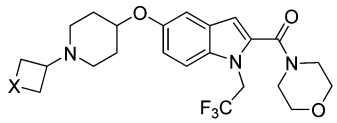
Table 1. cLogP, cLogD$_{7.4}$, and pK_a Values for More Common Saturated Heterocycles[d]

name	structure	cLogP[a]	LogD$_{7.4}$[a]	pK_a
azetidine		-0.183	-3.23	10.7[b]
pyrrolidine		0.376	-2.66	11.27[b]
piperidine		0.935	-2.1	11.2[b]
morpholine		-0.408	-2.65	8.4[b]
piperazine		-1.484	-3.58	9.8[b]
azepane		1.494	-1.53	10.89[c]
1,4-oxazepane		-0.377	-2.95	
1,4-diazepane		-0.925	-3.56	
oxetane		-0.163	-0.24	
tetrahydrofuran		0.526	0.33	
tetrahydropyran		0.955	0.89	

[a]See ref 24. [b]See ref 25. [c]See ref 26. [d]pK_a refers to the Het ⇆ HetH$^+$ equilibrium.

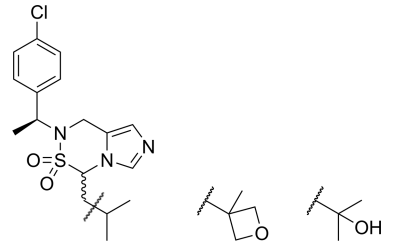
compound would be fully protonated and more polar than the oxetane analogue.



	X = O, **1**	X = CH$_2$, **2**
hH$_3$R EC$_{50}$ (nM) =	2	2
RLM CL$_{int}$ (μL/min/mg) =	228	28
LogD$_{7.4}$ =	1.83	2.09
pK_a =	6.4	9.1

Figure 1. In vitro potency and PK properties of selected H$_3$R inverse antagonists. The log $D_{7.4}$ and pK_a values were experimentally determined by Pierson et al.[27]

Another example where the oxetane was removed to improve the metabolic stability of the compound was found in the aldosterone synthase inhibitors from Adams et al. (see Figure 2).[28] Analogue **3** had IC$_{50}$ < 10 nM, but the isobutyl group was

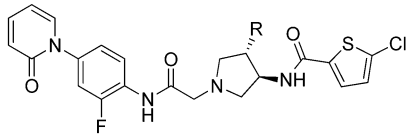


	3	**4**	**5**
hCYP11B2 IC$_{50}$ (nM) =	9.4	7.8	11.4
HLM $t_{½}$ (min) =	6	76	>300
cLogD$_{7.4}$ =	3.28	2.06	1.69

Figure 2. In vitro potency and PK properties of selected aldosterone synthase inhibitors.[28]

heavily metabolized. To mitigate this metabolism, the isobutyl group was replaced with a substituted oxetane (**4**), a compound with a more than 10-fold increase in metabolic stability. However, the metabolic stability was improved even further by replacing the oxetane ring with a *tert*-hydroxyl group (**5**). The decrease in cLogD$_{7.4}$ correlates with the improvement in metabolic stability for these three analogues.

Saturated Five-Membered Rings. In their factor Xa (FXa) program, Zbinden et al. added either a fluorine or hydroxyl group to a pyrrolidine ring in an attempt to improve metabolism (Figure 3).[29] The RLM clearance of pyrrolidine



	6	**7**	**8**
	R = H	R = F	R = OH
FXa K_i (nM) =	8	31	10
RLM CL$_{int}$ (μL/min.mg) =	79.2	65.9	15.8
cLogD$_{7.4}$ =	1.63	1.55	0.97
			Rat PK
			CL (mL/min/kg) = 74.0
			V_{ss} (L/kg)= 1.1
			%F = 38

Figure 3. In vitro potency and PK properties of selected FXa inhibitors.[29]

analogue **6** was only slightly improved by adding a fluorine to the ring (analogue **7**). However, when a hydroxyl group was added (**8**), the metabolic stability in RLM significantly improved probably because this compound was more polar than the other two. Unfortunately, analogue **8** had high rat in vivo clearance. After additional in vivo PK studies, Zbinden et al. found that 50% of **8** was cleared by the kidney unchanged.

Dragovich et al. also provided an example where replacing a pyrrolidine with a more polar ring improved metabolism. In their work on hepatitis C virus (HCV) NS5B inhibitors (Figure 4)[30] pyrrolidine analogue **9**, while potent against the intended target, had a short half-life of 10 min in human liver microsomes (HLM). Replacement of the pyrrolidine with the

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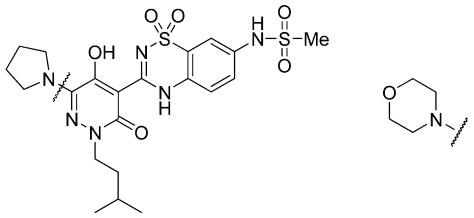
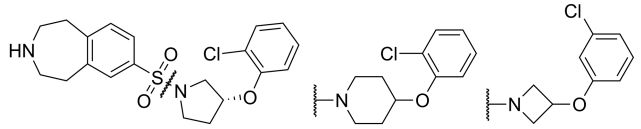


9	10
HCV replicon activity EC_{50} (μM) = 0.017	0.62
HLM $t_{½}$ (min) = 10	>60
$cLogD_{7.4}$ = -4.31	-5.29

Figure 4. In vitro potency and in vitro PKDM profile for selected HCV NS5B polymerase inhibitors.[30]

morpholine ring (**10**) lowered the $cLogD_{7.4}$ and significantly improved the metabolic stability.

Other groups have demonstrated that changing the ring size of the pyrrolidine can reduce metabolism. For example, in their serotonin receptor subtype 2C (5-HT_{2C}) agonist program, Fish et al. changed the pyrrolidine in one of their analogues to a piperidine and an azetidine (Figure 5).[31] The piperidine

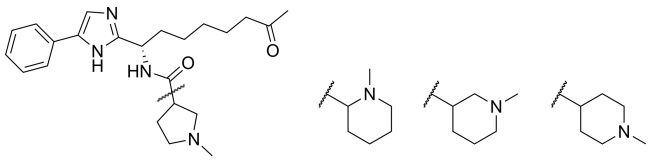


11	12	13
5-HT_{2C} EC_{50}, E_{max} (nM, %) = 14, 73	31, 66	12, 82
$LogD_{7.4}$ = 1.6	2.0	1.7
HLM CL_{int} (μL/min/mg) = 36	17	9

Figure 5. In vitro potency and in vitro PKDM profile for selected 5-HT_{2C} agonists. The log $D_{7.4}$ values were experimental determined by Fish et al.[31]

analogue **12** showed a slight improvement in HLM stability over the pyrrolidine **11**, while the azetidine analogue **13** was the most stable. The position of the chloride on the phenyl ring was also modified on azetidine analogue **13** so that change may have also influenced the metabolic stability.

Pescatore et al. also prepared six-membered ring analogues of their pyrrolidine containing compound to improve metabolism (Figure 6).[32] In their anticancer program on histone deacetylase 1 (HDAC1) inhibitors, the analogue that contained a pyrrolidine ring, **14**, had a RLM intrinsic clearance (CL_{int}) of >500 $\mu L\ min^{-1}\ mg^{-1}$. By replacing this group with various piperidine analogues, they identified a compound with a greater than 7-fold improvement in RLM stability (see compound **17**).



14	15	16	17
HDAC1 IC_{50} (nM) = 280	710	220	250
RLM, CL_{int} (μL/min/mg) = 501	>300	154	68
$cLogD_{7.4}$ = 0.66	2.54	1.55	1.49

Figure 6. In vitro potency and RLM data for selected HDAC1 inhibitors.[32]

Interestingly, the most stable piperidine analogue **17** had a higher $cLogD_{7.4}$ than **14**, suggesting that clearance was not driven entirely by the lipophilicity of the compound.

Saturated Six-Membered Ring Heterocycles. To improve the metabolism of their piperidine analogues, Wan et al. employed a strategy of substituting the piperidine with a fluorine or adding polar groups to the ring (Figure 7).[33] In their 11β-hydroxysteroid dehydrogenase type I (11β-HSD1) program, they found that their compound containing the unsubstituted piperidine, **18**, had a $t_{1/2}$ of 6 min in mouse liver microsomes (MLM). The analogues with polar groups added to the ring were all more stable in MLM, with 4-fluoro analogue (**20**), thiomorpholine 1,1-dioxide (**22**), 4-carboxyl (**23**) having MLM $t_{1/2}$ > 30 min. Analogues **22** and **23** are the least lipophilic of this set of compounds, which may explain why they have better metabolic stability than analogues **18**, **19**, and **21**. The improved metabolic stability of the 4-fluoropiperidine analogue **20** suggests that the fluorine atom may have blocked a site of metabolism that was present on parent compound **18**. Carboxyl analogue **23** was also efficacious in an in vivo PD model that measured 11β-HSD1 inhibition in epididymal fat (40% inhibition at 10 mg/kg, po).

In their anticancer program on aurora kinase inhibitors, Kerekes and co-workers improved the PK profile of their analogues containing piperidine by adding fluorine atoms and altering the ring size (Figure 8).[34] One of their more advanced compounds in the paper contained a 4-fluoropiperidine (**24**), which had a rat oral AUC of 2.2 μM·h. To further improve the PK, the 4,4-difluoro analogue (**25**) was prepared, but it had a lower rat oral AUC. The five- and four-membered ring difluoro analogues **26** and **27**, respectively, had higher oral AUC than the six-membered ring analogue **25**. The four-membered ring analogue **27** had the highest oral AUC, but Kerekes et al. discovered that the azetidine ring was prone to nucleophilic ring-opening. Turning to the five-membered ring analogue **26**, Kerekes et al. discovered that a major route of metabolism in human and monkey hepatocytes was oxidation of the benzylic position adjacent to the isothiazole ring. To circumvent this problem, they deuterated the benzylic position of the 3,3-difluoropyrrolidine, which led to a compound (**28**) that had superior rat iv clearance, oral AUC, and bioavailability. This compound was advanced into a mouse tumor xenograft PD assay, where it reduced the phosphorylation of histone H3 (HH3) in the tumor by ~80% after being dosed orally at 100 mg/kg. The addition of deuterium atoms to mitigate metabolism takes advantage of the kinetic isotope effect, which increase the energy required to fragment the carbon–deuterium bond.[35] This approach of incorporating deuterium to improve metabolism has gained renewed interest among medicinal chemists in the past few years.[36]

Another example where fluorine atoms were added to the piperidine ring was reported by Gleave et al. (Figure 9).[37] In their cannabinoid receptor 2 (CB2) agonist program, they had tested the metabolic stability in RLM for a piperidine containing analogue (**29**) and its 4,4-difluoro derivative (**30**). There was very little improvement in the RLM metabolic stability for the 4,4-difluoro analogue. However, when the piperidine ring was replaced with the more polar morpholine ring (**31**), the metabolic stability improved by 10-fold in RLM. This trend in improved metabolic stability followed the reduction in $cLogD_{7.4}$.

Peglion and co-workers were able to improve the metabolic stability of their analogue containing a piperidine by adding a

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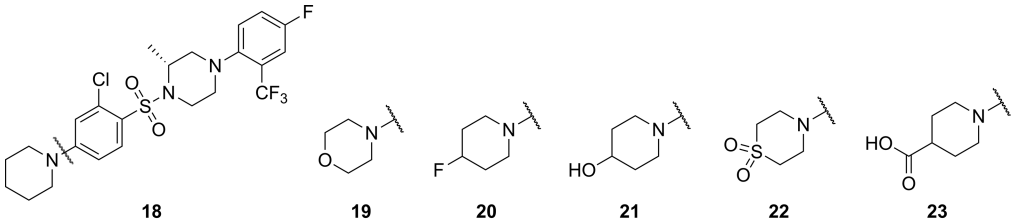
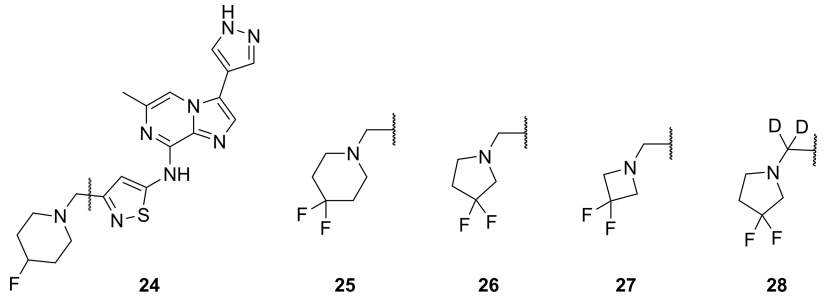


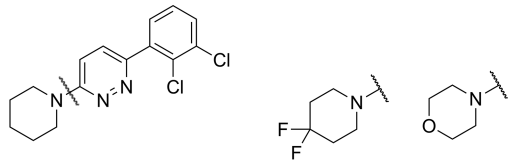
	18	19	20	21	22	23
11β-HSD1 IC_{50} (nM) = 97		10	50	10	10	10
MLM $t_{1/2}$ (min) = 6		14	>30	14	>30	>30
$cLogD_{7.4}$ = 5.90		4.28	5.35	4.37	3.08	1.97
		mouse PK				mouse PK
		CL (mL/min/kg) = 55				9
		V_{ss} (L/kg)= 4.5				2.0
		$t_{1/2}$ (h) = 2.1				3.5
		%F = 12				100

Figure 7. In vitro potency and PK properties of selected 11β-HSD1 inhibitors. PK experiments were conducted with C57B6 mice at 2 mg/kg (iv) and at 10 mg/kg (for **23**) or 30 mg/kg (for **19**) (po).[33]



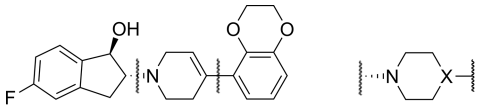
	24	25	26	27	28
	Rat PK				
PO AUC (μM·h) = 2.2		0.95	1.61	12.07	4.75
IV CL (mL/min)/kg = 33					6.3
%F = 19					33

Figure 8. Rat PK of selected aurora kinase inhibitors.[34] All compounds had IC_{50} at Aurora A and Aurora B of ≤4 and ≤13 nM, respectively. The po AUC was determined in SD rats from 0−6 h after a 10 mg/kg oral dose. The iv PK was run at 10 mg/kg and % *F* determined from 30 mg/kg po.



	29	30	31
CB_2 pEC_{50} (Efficacy) = 8.2 (86%)		7.8 (83%)	7.1 (76%)
Rat CL_{int} (mL/min/kg) = 35		29	2.8
$cLogD_{7.4}$ = 3.8		2.9	2.2

Figure 9. In vitro potency and metabolic stability of selected CB2 agonists.[37]



	32	33	34
		X = CH	X = N
5-HT_{1A} pK_i = 9.07		9.36	8.90
MF% = 8		7	65
$cLogD_{7.4}$ = 3.02		2.55	2.51

Figure 10. In vitro potency and metabolic stability for selected 5-HT_{1A} receptor agonists.[38] The metabolic bioavailability prediction (MF%) was determined from the stability of test compound (concentration of 0.1 μM) in human liver microsomes where MF% = 100% is equal to no metabolism.

nitrogen (Figure 10).[38] In their 5-HT_{1A} agonist program for the treatment of anxiety and depression, the 1,2,3,6-tetrahydropyridine analogue (**32**) and the piperidine analogue (**33**) both had poor metabolic stability (MF% of 8 and 7, respectively). However, the corresponding piperazine (**34**) was nearly 10-fold more metabolically stable than the piperidine analogue. The difference in $cLogD_{7.4}$ of compounds **33** and **34** is small, so the improved metabolic stability observed with compound **34** cannot be explained by a reduction in lipophilicity. Perhaps the C-4 position of the piperidine in compound **33** is a site for metabolism, which would be blocked by the piperazine nitrogen in analogue **34**. This piperazine analogue was active in a rodent ultrasonic vocalization test, which is an animal model for anxiety.

The piperidine analogues reported by Qiao et al. from their erythropoietin-producing hepatocellular carcinoma receptor type B3 (EphB3) inhibitor program illustrate how the position of the nitrogen atom can affect metabolism (Figure 11).[39] Their 1-substituted piperidine analogue **35** was significantly less stable in MLM than the analogue with the 4-substituted piperidine (**36**). This change in the position of the nitrogen lowered the $cLogD_{7.4}$ and produced an analogue with a more basic nitrogen, which may have contributed to the improvement in metabolic stability. This compound, named LDN-211904[39] (**36**), was one of their lead compounds for their EphB3 inhibitor program.

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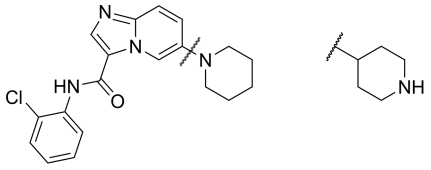
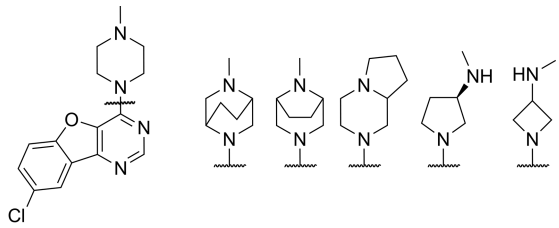


	35	LDN-211904, 36
EphB3 IC_{50} (nM) =	87	79
$t_{½}$ (min) =	20	348
CL_{int} (μL/min/mg) =	71	4
$cLogD_{7.4}$ =	3.44	0.14

Figure 11. In vitro potency and metabolic stability of selected EphB3 inhibitors. Half-life and CL_{int} were determined in MLM.[39]

An example where a piperazine ring was modified to improve metabolism was found in the report from Cramp et al. on their H4R antagonists (Figure 12).[40] *N*-Methylpiperazine analogue



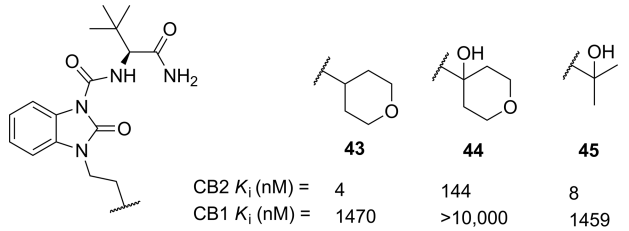
	37	38	39	40	41	42
hH4R IC_{50} (nM) =	19	169	440	236	33	87
RLM % remain. =	1	66	62	0	92	89
$cLogD_{7.4}$ =	2.35	2.30	1.74	2.60	0.01	0.96

Figure 12. In vitro potency and RLM stability for selected human H4R inverse agonists. The percent remaining in RLM was measured after a 10 min incubation.[40]

37 was found to undergo rapid N-dealkylation in RLM, so a number of analogues were prepared to circumvent this problem. The bridged piperazine analogue **38** had a similar $cLogD_{7.4}$, but the RLM was significantly improved, suggesting that the increase in sterics surrounding the site of metabolism may have been responsible for the lower turnover. Another bridged piperazine analogue (**39**) had similar stability to **38**, but the analogue containing the 5,6-ring (**40**) was completely eliminated by RLM. Interestingly, the analogues containing the exocyclic aminomethyl group, pyrrolidine **41** and azetidine **42**, were the most metabolically stable in this set. The large reduction in $cLogD_{7.4}$ may explain why these two analogues were more stable than the other compounds in this series.

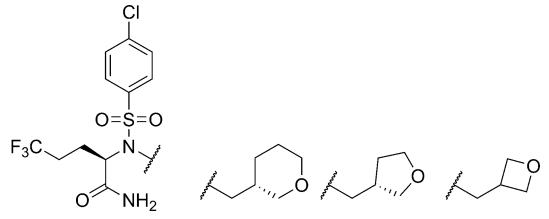
Turning to examples with a THP ring, Omura and co-workers were able to improve the metabolic stability of their CB2 agonist by adding a hydroxyl group to the 4-position of the THP ring (Figure 13).[41] The hydroxylated THP analogue **44** was nearly 6-fold more metabolically stable in HLM than the unsubstituted THP analogue **43**. While **44** was less potent than the parent THP analogue, the principle of having a tertiary alcohol at that position to improve metabolic stability was applied to the lead compound in their paper (**45**), which had an HLM $t_{1/2}$ of >120 min and was nearly as potent as **43**.

Another example with THP rings was illustrated in the γ-secretase inhibitor program from Stepan et al. (Figure 14).[42] In this paper they showed that decreasing the ring size of the THP in their inhibitors improved the metabolic stability. Stability in HLM improved going from the THP (**46**) to THF (**47**) to the oxetane analogue (**48**). The authors point out that the



	43	44	45
CB2 K_i (nM) =	4	144	8
CB1 K_i (nM) =	1470	>10,000	1459
HLM $t_{½}$ (min) =	15	84	>120
$cLogD_{7.4}$ =	0.94	-0.04	0.61

Figure 13. In vitro potency and metabolic stability of selected CB2 agonists.[41]

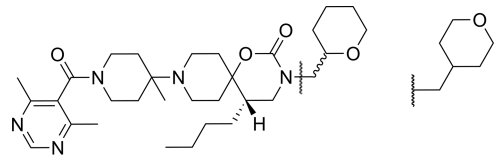


	46	47	48
IC_{50} ($Aβ_{42}$, nM) =	45.2	11.2	16.9
Notch selectivity =	103	363	287
HLM $CL_{int, app}$ (mL/min/kg) =	136	69.8	28.6
$LogD_{7.4}$ =	3.70	2.80	2.50

Figure 14. In vitro potency and metabolic stability of selected γ-secretase inhibitors.[42] The log $D_{7.4}$ was determined experimentally by Stepan et al. using the method of Lombardo et al.[43]

increased metabolic stability was most likely a result of the reduction in log $D_{7.4}$. However, they also suggest that the reduced C−H bond reactivity of the oxetane ring compared to the THP and THF rings may have also contributed to the improved metabolic stability.

The position of the oxygen atom in the ring can also affect the metabolism of a THP ring. In their work on C−C chemokine receptor type 5 (CCR5) receptor antagonists containing THP rings (Figure 15), Rotstein et al. found that



	49	50
CCR5 IC_{50} (nM) =	10	25
HLM CL (μL/min/mg) =	53	12
$cLogD_{7.4}$ =	2.33	2.22

Figure 15. In vitro potency and metabolic stability of selected CCR5 receptor antagonists.[44]

the 4-substituted-THP analogue was 4-fold more stable in HLM than the 2-substituted-THP derivative (cf., **49** vs **50**).[44] These two regiochemical isomers share nearly identical properties except for the position of the oxygen in the THP ring, so the metabolic differences cannot be explained by large difference in physical properties. The 4-THP analogue **50** was advanced into rat, dog, and monkey PK and also had IC_{50} = 0.18 nM in a PBMC (peripheral blood mononuclear cell) viral replication assay.

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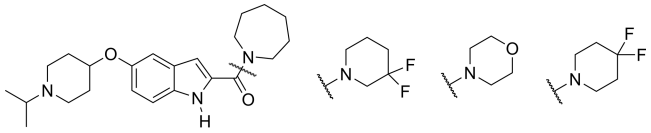
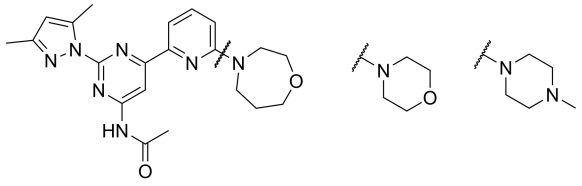
Saturated Seven-Membered Ring Heterocycles. Seven-membered ring heterocycles are generally more lipophilic than their smaller ring counterparts, so methods for improving the metabolic stability of these rings typically involve reducing the ring size. For example, in the H_3R inverse agonists work from Pierson et al., they found that their analogue containing an azepane ring (**51**) had a RLM clearance (CL) of 55 μL min^{-1} mg^{-1} (Figure 16).[27,45] Replacing the ring with difluoropiperidine rings (**52**, **54**) or morpholine (**53**) produced analogues with improved metabolic stability. In this case, a combination of reducing ring size and adding fluorine atoms or polarity was used to reduce the metabolism.



	51	**52**	**53**	**54**
hH_3R (nM) K_i =	6	2	5	2
RLM (μL/min/mg) =	55	17	12	9
$LogD_{7.4}$ =	1.75	1.23	0.09	1.2
Rat in vivo PK				
CL_{int} (mL/min/kg) =		50		18
V_{ss} (L/kg) =		3.5		6
$t_{½}$ (h) =		1.1		4
%F =				100

Figure 16. In vitro potency and PK properties of selected H_3R inverse agonists. In vivo PK experiments were run with Wistar rats at 1 mL/kg (iv) and 4 mL/kg (po). The log $D_{7.4}$ values were experimentally determined by Pierson et al.[27,45]

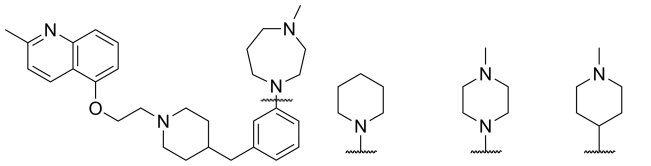
Zhang and co-workers gave an example of improving the metabolic stability of a seven-membered 1,4-oxazepane ring (Figure 17). In their adenosine type 2A (A_{2A}) receptor antagonist program for Parkinson's disease, they found that reducing the ring size of the 1,4-oxazepane in analogue **55** with either a morpholine (**56**) or an *N*-methylpiperazine ring (**57**) greatly improved metabolic stability.[46]



	55	**56**	**57**
hA_{2A} K_i (nM) =	2.3	0.83	0.71
hA_1 K_i (nM) =	280	130	120
RLM CL_{int} (mL/min kg) =	1000	25	25
HLM CL_{int} (mL/min kg) =	94	3.0	3.0
$cLogD_{7.4}$ =	-0.46	-0.78	-0.54

Figure 17. In vitro potency and metabolic stability for selected human A_{2A} receptor antagonists.[46]

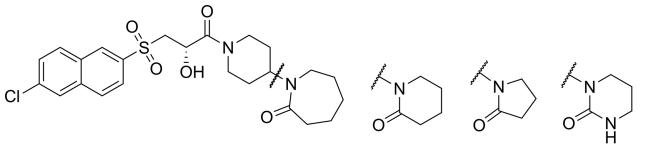
Ward et al. showed how the metabolic stability of a 1,4-diazepane was improved by reducing the ring size (Figure 18).[47] In their 5-HT_1 receptor ligand program, the metabolism of the analogue containing the 1,4-diazepane (**58**) was greatly improved by replacing the ring with piperidine rings (**59**, **61**) or a piperazine ring (**60**). While the *N*-methylpiperazine derivative (**60**) and *N*-methylpiperidine derivative (**61**) had similar stability in RLM, **61** had lower in vivo rat clearance. In this set of compounds there is no correlation of $cLogD_{7.4}$ with metabolic stability, suggesting the ring size had more of an influence on metabolism.



	58	**59**	**60**	**61**
5-$HT_{1A,1B,1D}$ pK_i =	8.6, 8.1, 9.2	8.4, 8.7, 9.0	9.0, 8.2, 9.3	8.5, 8.3, 9.7
RLM (mL/min/g) =	26	6.2	2.9	2.0
$cLogD_{7.4}$ =	2.85	5.43	3.87	2.89
Rat in vivo PK				
CL (mL/min/kg) =		46		14
V_{ss} (L/kg) =		4.4		12.4
$t_{½}$ (h) =		1.3		12

Figure 18. In vitro potency and metabolic profile for selected 5-$HT_{1A,B,D}$ receptor ligands.[47] Rat in vivo PK was determined after 1 mg/kg dosing iv.

In their work on FXa inhibitors for the treatment of thrombotic disease, Fujimoto et al. had an analogue that contained the seven-membered ring caprolactam (**62**) (Figure 19).[48] The metabolic stability was progressively improved by replacing this ring with the six- and five-membered ring lactams (**63** and **64**). The six-membered ring cyclic urea analogue **65** had the best metabolic stability of this set and was advanced into the clinic as TAK-422.[48] The reduction in metabolism follows the trend of reduced lipophilicity for this set of compounds as noted by the reduction in $cLogD_{7.4}$.



	62	**63**	**64**	**65** (TAK-422)
FXa IC_{50} (nM) =	28	9.1	14	3.5
$LogD_{7.4}$ =	2.52	2.19	1.96	1.81
MoLM % eliminated =	100	77.2	33.2	16.4
HLM % eliminated =	91.2	31.6	8.0	6.4

Figure 19. In vitro potency and metabolic stability for selected FXa inhibitors. The percent eliminated data were determined after 20 min incubations with NADPH and LM. The log $D_{7.4}$ data were determined experimentally by Fujimoto et al.[48] (MoLM = monkey liver microsomes).

In their anticancer program, Tsou et al. prepared a mammalian target of rapamycin (mTOR) inhibitor containing an aza-bicyclo seven-membered ring (**66**) (Figure 20).[49] This compound was rapidly metabolized by both phase I and phase II metabolism in MLM. However, reducing the ring size of the seven-membered ring with either a morpholine or *N*-methylpiperazine ring led to analogues (**67** and **68**) with greatly improved metabolic stability. Since in vivo studies for mTOR inhibitors typically involve the use of nude mice, the MLM studies were conducted with microsomes from this strain, although a recent report from Martignoni et al.[50] showed that there are no significant metabolic differences between MLM from normal (CD-1) mice and nude mice.

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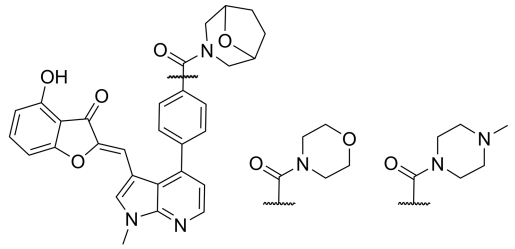
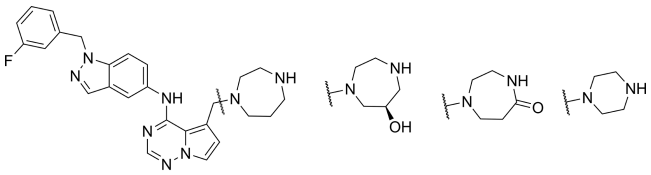


66	67	68
mTOR IC_{50} (nM) = 0.56	0.5	1
MLM (Phase I) $t_{½}$ (min) = 6	>30	28
MLM (Phase I/II) $t_{½}$ (min) = 2	10	24
$cLogD_{7.4}$ = 3.20	2.78	3.14

Figure 20. In vitro potency and metabolic stability for selected mTOR inhibitors. MLM studies were conducted with MLM from nude mice. Phase I metabolism studies were conducted with NADPH, while phase I/II studies were conducted with both NADPH and uridine 5′-diphosphoglucuronic acid (UDPGA).[49]

Mastalerz et al., in their work on human epidermal growth factor receptor 2/epidermal growth factor receptor (HER2/EGFR) dual inhibitors for the treatment of cancer, tried to improve the metabolic stability of their analogue containing a 1,4-diazepane (**69**) by adding polar groups to the ring (Figure 21).[51] However, adding a hydroxyl or carbonyl to the 1,4-



69	70	71	72
HER2 IC_{50} (nM) = 27	16	75	81
EGFR IC_{50} (nM) = 33	26	240	90
N87 cell assay IC_{50} (nM) = 110	160	200	730
MLM CL (mmol/min/mg prn) = 0.05	0.12	0.27	0
$cLogD_{7.4}$ = -0.57	-1.12	0.41	0.11

Figure 21. In vitro potency and metabolic stability of selected dual EGFR/HER2 inhibitors.[51]

diazepane (**70** and **71**) ring gave analogues that were both metabolically less stable than the parent analogue **69**. Ultimately, the analogue with the best microsomal stability was the six-membered piperazine analogue **72**. Here again, reducing the ring size improved metabolic stability.

HETEROAROMATIC COMPOUNDS

Five-Membered Heteroarenes. Five-membered heteroarenes are commonly used in medicinal chemistry programs and are used as bioisosteres for carboxamides, esters, and carboxylic acids.[9] However, because of the electron-rich nature of the ring, they are prone to undergo oxidative metabolism, which leads to electrophilic species.[13] The inherent electrophilicity of these metabolites is thought to be in part responsible for the idiosyncratic toxicity of some drugs containing five-membered heteroarenes.[18,19] That being said, medicinal chemists have identified methods for reducing the metabolism of these rings by adding another nitrogen to the ring, blocking a potential site of metabolism with a fluorine, or replacing the ring with another heterocycle. Examples of each of these methods as well as others are summarized below. As with the saturated heterocycles, we have tabulated some of the calculated and measured physical properties for some of the more common five-membered ring heterocycles (Table 2). In addition to cLogP, $cLogD_{7.4}$, and pK_a, we have included the ionization potential (IP) as a measure of the electron-rich nature of the ring. As elegantly summarized in the review by Dalvie et al.,[13] all of these properties can influence the metabolic stability of five-membered heteroarenes.

Table 2. cLogP, $cLogD_{7.4}$, pK_a, and Ionization Potentials for Some Common Five-Membered Ring Heteroaromatics[c]

name	structure	cLogP[a]	$LogD_{7.4}$[a]	pK_a[b]	IP (eV)[b]
pyrrole		0.748	0.75	-3.8	8.23
imidazole		-0.029	-0.5	7	8.78
pyrazole		0.241	0.32	2.52	9.15
1,2,3-triazole		-0.186	0.21	1.17, 9.3	10.06
1,2,4-triazole		-0.281	-0.58	2.19, 10.26	10
tetrazole		-0.463	-2.47	-3.0, 4.89	11.3
furan		1.318	1.38		8.89
oxazole		-0.179	0.12	0.8	9.83
isoxazole		0.121	0.08	-2.97	10.17
1,2,4-oxadiazole		-0.506	-0.74		
1,3,4-oxadiazole		-1.406	-0.74		
1,2,5-oxadiazole		0.164	-0.25	-5	11.79
thiophene		1.788	1.9		8.87
thiazole		0.486	0.44	2.52	9.5
isothiazole		0.871	0.81	-0.51	9.42

[a]See ref 24. [b]See ref 25. [c]pK_a refers to the Het ⇆ $HetH^+$ equilibrium. For the triazoles and tetrazole, the second pK_a refers to the HetH ⇆ Het^- equilibrium.

Geng et al. in their work on glutamate racemase (MurI) inhibitors for *H. pylori* found that adding nitrogen atoms to the electron-rich thiophene and furan rings significantly improved metabolic stability (Figure 22).[52] The high intrinsic clearance in both HLM and MLM of their thiophene analogue **73** was mitigated by replacing the ring with either a thiazole (**74**) or isothiazole (**75**), and the metabolic stability was improved when the furan on analogue **76** was replaced with an oxazole (**77**). The more metabolic stable analogues were all more polar than the analogue **73**.

In their CB2 agonist program, Riether et al. improved the metabolic stability of their compound that contained a thiazole

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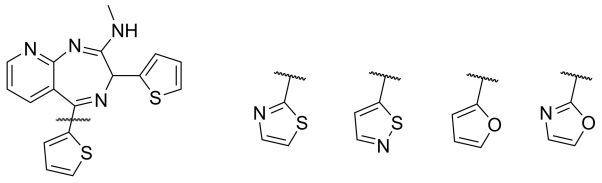
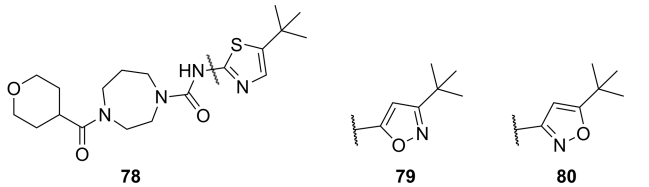


	73	74	75	76	77
MurI IC_{50} (μM) = 0.7		1.7	1.1	2.0	7.7
HLM CL_{int} (μL/min/mg) = 30		<14	18	13	<5
MLM CL_{int} (μL/min/mg) = >100		33	37	30	19
$cLogD_{7.4}$ = 1.41		0.20	0.33	0.89	-0.38

Figure 22. In vitro potency, HLM, and MLM data for selected MurI inhibitors.[52]

ring (analogue **78**, Figure 23),[53] by replacing it with isoxazole rings (analogues **79** and **80**). A decrease in lipophilicity cannot



	78	79	80
CB2 EC_{50} (nM) (efficacy) = 150 (106%)		26 (99%)	67 (104%)
HLM $t_{½}$ (min) = 27		>120	>120
$cLogD_{7.4}$ = 0.77		0.98	1.75

Rat in vivo PK
CL = 34 mL/min/kg
V_{ss} = 0.87 L/kg
%F = 28

Figure 23. In vitro potency and HLM data for select CB2 agonists.[53] All compounds displayed weak activity against CB1 (EC_{50} > 20 μM). Rat in vivo PK experiments were performed using Wistar rats at 1 μmol/kg (iv) and 10 μmol/kg (po).

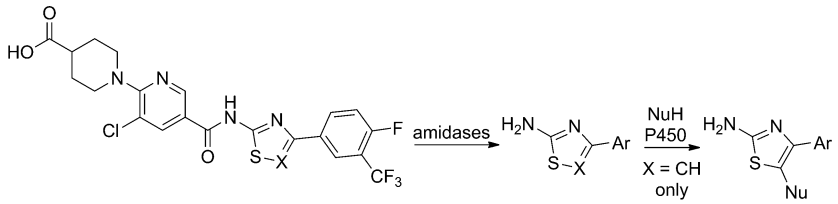
explain why isoxazole analogues **79** and **80** were more metabolically stable than thiazole analogue **78**, since the $cLogD_{7.4}$ is higher for analogues **79** and **80**. If metabolism occurs on the five-membered heteroarene, the improved metabolic stability of **79** and **80** might be explained by the fact that the isoxazole ring is less electron-rich than the thiazole ring, as noted by the higher ionization potential for isoxazole.

In their antithrombocytopenia program, Kalgutkar et al. found that their thrombopoietin (Tpo) receptor agonist containing a thiazole (**81**) underwent amide hydrolysis to form an intermediate (**84**), which was prone to bioactivation and attack of nucleophiles at the C-5 position (Figure 24).[54] However, incorporation of a fluorine (**82**) or nitrogen (**83**) at the C-5 position of the thiazole prevented the bioactivation pathway from occurring. The rat in vivo PK for these two new analogues was also better than the parent compound. Interestingly, the $cLogD_{7.4}$ of the two new compounds was higher than the parent compound. Nevertheless, the strategic blocking of the site of bioactivation mitigated the formation of the reactive intermediates.

In a recent report from Obach et al., they found that the metabolic profile of a thiazole ring dramatically changed when the ring was substituted with a methyl group (Figure 25).[55] The cyclooxygenase (COX-1/2) inhibitors sudoxicam (**86**) and meloxicam (**87**) have the same structure except that meloxicam has a methyl group at the C-5 position of the thiazole ring. P450 mediated oxidation of sudoxicam occurred to form a reactive epoxide **88**, which after hydrolysis produced the protoxin acylthiourea **89**. In contrast, the major route of metabolism for meloxicam involved oxidation of the methyl group to give metabolites **90** and **91**, which did not form reactive species. The protoxin acylthiourea **89**, formed during the metabolism of sudoxicam, may in part be responsible for the hepatotoxicity observed with sudoxicam.[56] Meloxicam, on the other hand, is not metabolized to a protoxin and has not been associated with hepatotoxicity.[57,58] As pointed out in the previous example, the C-5 position is a "soft spot" for bioactivation of the thiazole ring, so the methyl group on meloxicam serves the purpose of blocking this site as well as providing an alternative site for metabolism.

In the work by Ioannidis and co-workers on Janus kinase 2 (JAK2) inhibitors, they showed that the metabolic stability was better for their analogue containing a pyrazole (**93**) rather than a thiazole ring (**92**) (Figure 26).[59] While the difference in $cLogD_{7.4}$ between these two rings cannot explain the improvement in metabolism, pyrazole rings are known to be relatively stable to metabolism compared to other five-membered ring heteroaromatic compounds.[13]

Another example where the pyrazole ring was employed to improve metabolic stability was illustrated in the work from Tremblay et al. in their Hedgehog (Hh) pathway antagonists for the treatment of cancer (Figure 27).[60] Compound **94** was



	X = CH, **81**	X = CF, **82**	X = N, **83**
Rat PK			
IV CL_p (mL/min/kg) = 6.89		2.79	1.49
IV V_{ss} (L/kg) = 0.83		0.34	0.22
IV $t_{½}$ (h) = 1.34		1.67	1.09
%F = 66.8		73.0	73.5
$cLogD_{7.4}$ = 1.28		1.72	2.58

84 **85**

Figure 24. In vivo PK data for selected Tpo receptor antagonists. NuH is protein nucleophile or glutathione (GSH). In vivo PK was measured in male SD rats (1 mg/kg iv, 5 mg/kg po).[54]

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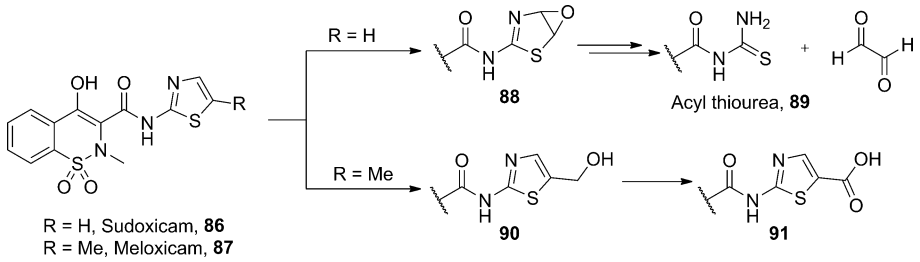
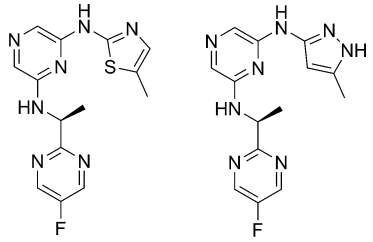
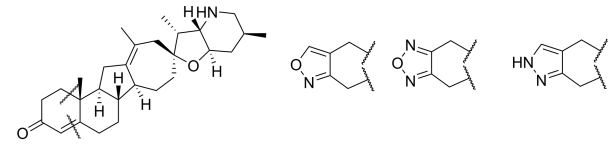


Figure 25. Metabolism of sudoxicam and meloxicam.[55]



	92	93
JAK2 IC_{50} (μM) = 0.004		< 0.003
RLM CL_{int} (μL/min/mg) = 52		< 4
$cLogD_{7.4}$ = 1.44		1.78

Figure 26. In vitro potency and RLM stability for selected JAK2 inhibitors.[59]

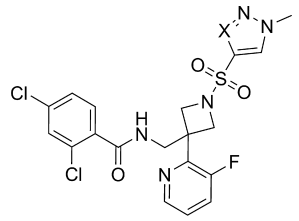


	94	95	96	97
Cellular Hh inhibition EC_{50} (μM) = 0.30		0.22	0.23	0.013
HLM, $t_{½}$ (min) = 75		8	8	120
$cLogD_{7.4}$ = 2.90		4.19	3.87	4.44
Rat PK				
IV CL (mL/min/kg) = 7.5				
IV V_{ss} (L/kg) = 13				
IV $t_{½}$ (h) = 1.2				
%F = 34				

Figure 27. In vitro potency and HLM data for selected orally active Hh pathway antagonists. In vivo PK experiments were conducted with SD rats (iv dose of 1 mg/kg, po dose of 5 mg/kg).[60]

one of the initial leads in their program, but in monkey PK studies, the α,β-unsaturated ketone present in **94** underwent metabolism to give the corresponding saturated alcohol. Part of the strategy to circumvent this problem was to reduce the double bond and replace the ketone with five-membered heteroarenes. The pyrazole containing analogue **97** showed a dramatic improvement in metabolic stability (HLM $t_{1/2}$ = 120 min), but the isoxazole analogue **95** and 1,2,5-oxadiazole analogue **96** were less metabolically stable. A decrease in lipophilicity cannot explain the improvement in metabolic stability, since the most metabolically stable compound **97** had the highest $cLogD_{7.4}$. If metabolism occurred on the isoxazole ring of analogue **95**, the decrease in metabolic stability might be explained by the potential for this ring to undergo metabolically mediated ring cleavage, which is precedented in the literature.[61] The poor metabolic stability of analogue **96** is less easily explained, since little has been reported on the metabolism of 1,2,5-oxadiazole rings.[62]

Another example of a compound containing a pyrazole was found in the work on glycine transporter 1 (GlyT1) inhibitors from Thomson and co-workers (Figure 28).[63] The pyrazole

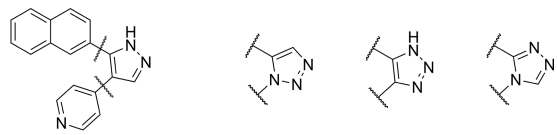


	98	99
	X = CH	X = N
hGlyT1 IC_{50} (nM) = 2.95		0.31
RLM (%TO) = 56		15
DLM (%TO) < 5		5
HLM (%TO) = 25		22
$cLogD_{7.4}$ = 1.83		0.88

Figure 28. In vitro potency and LM data for selected GlyT1 receptor antagonists[63] (%TO = percent turnover).

ring analogue **98** was replaced with a 1,2,3-triazole ring (**99**), which had little effect on improving dog liver microsomes (DLM) and HLM stability, but it had improved RLM stability. The improved RLM stability might be explained by the lower lipophilicity of analogue **99**.

Nietz and co-workers, in their work on c-Jun N-terminal kinase 3 (JNK3) inhibitors, also compared the microsomal stability of analogues containing a pyrazole with various triazoles (Figure 29).[64] The pyrazole analogue **100** had poor



	100	101	102	103
JNK3 IC_{50} (μM) = 0.245		4.81	0.364	4.48
MLM (% remaining at 30 min) = 1		22	45	76
glucuronidation (% remaining at 60 min) = 100		90	nd	87
$cLogD_{7.4}$ = 3.57		3.30	2.58	3.51

Figure 29. In vitro potency, MLM, and glucuronidation data for select JNK inhibitors.[64]

metabolic stability in MLM. Replacing the pyrazole central core with a triazole had differing effects on metabolism, depending on which regioisomer of the triazole ring was used. Two analogues containing the 1,2,3-triazole (**101**, **102**) showed an improvement in MLM stability, but the 1,2,4-triazole analogue **103** was the most metabolically stable. Why the 1,2,4-triazole analogue **103** was more stable than 1,2,3-triazole analogues is difficult to explain, especially since there was little difference in the lipophilicity between analogues **100** and **103**. Since some triazoles are CYP P450 inhibitors,[13] the improved metabolic stability of **101**−**103** might be explained by their ability to inhibit these metabolizing enzymes. Unfortunately, CYP P450 inhibition data were not disclosed for this set of compounds. Triazole containing compounds are also known to undergo

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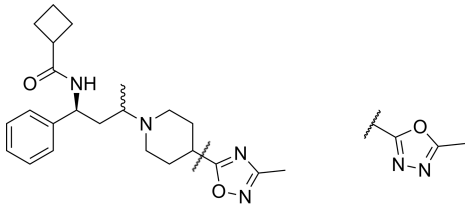
elimination through glucuronidation,[13] but analogues **101** and **102** were both relatively stable in a glucuronidation assay.

Another example where the position of three heteroatoms in the ring had a significant effect on metabolism was reported by Barber et al. on the CCR5 program for HIV (Figure 30).[65]

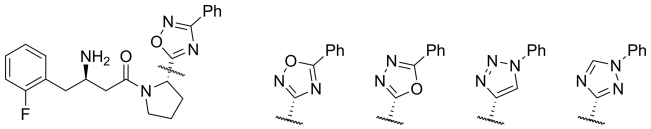


	104	**105**
Fusion IC_{50} (nM) = 21.4		1849
HLM (μL/min/mg) = 56		<7
$LogD_{7.4}$ = 2.1		1.3

Figure 30. In vitro potency and HLM data for selected CCR5 antagonists. The log $D_{7.4}$ values were experimentally determined by Barber et al.[65]

Their analogue with the 1,2,4-oxadiazole **104** was less metabolically stable than the 1,3,4-oxadiazole analogue **105** in HLM. Compound **105** is more polar than analogue **104**, which may explain the improvement in metabolic stability. Also, if the five-membered ring on compound **104** is the site of metabolism, the 1,2,4-oxadiazole ring may undergo metabolic N−O ring-opening, which is precedented in the literature.[13,66] Without an N−O bond, the 1,3,4-oxadiazole ring on **105** would not undergo this route of metabolism.[67]

In their work on dipeptidyl peptidase IV (DPP-4) inhibitors for the treatment of type 2 diabetes, Nordhoff et al. determined the metabolic stability of a number of three-heteroatom five-membered heterocycles (Figure 31).[68] The orientations of the

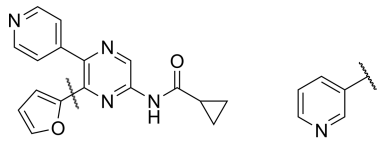


	106	**107**	**108**	**109**	**110**
DPP-4 IC_{50} (μM) = 0.031		0.090	0.120	0.130	0.062
HLM (% metabolized @ 1 h) = 38		24	16	13	1
RLM (% metabolized @ 1 h) = 89		81	33	36	18
$cLogD_{7.4}$ = 2.63		1.50	0.92	2.15	1.93

Figure 31. In vitro potency and LM data for selected DPP-4 inhibitors.[68]

heteroatoms within these rings were important factors for metabolic stability. The lower lipophilicity of the 1,3,4-oxadiazole analogue **108** might explain why it was more metabolically stable than the 1,2,4-oxadiazole analogues **106** and **107**. Also, as pointed out in the previous example,[65] if the five-membered heteroarene is the site of metabolism, the 1,2,4-oxadiazole analogues might be susceptible to metabolic ring-opening. The improvement in metabolic stability of the 1,2,4-triazole analogue **110** over the 1,2,3-triazole analogue **109** might in part be explained by the slight decrease in lipophilicity of analogue **110**.

All of the previous examples involved replacing one five-membered heteroarene for another. However, another strategy for reducing the metabolism of a five-membered heteroarene is to replace it with a six-membered heteroaromatic ring, which tends to be more electron poor than a five-membered heteroarene. Eastwood et al. illustrated this method in their A_{2B} receptor antagonists program where they replaced a furan ring for a pyridine ring (Figure 32).[69] The metabolic stability of



	111	**112**
hA_{2B} K_i (nM) = 2.2		25
%TO RLM/HLM = 46/18		20/14
$cLogD_{7.4}$ = 2.3		1.79

Figure 32. In vitro potency and microsomal stability for selected A_{2B} receptor antagonists.[69]

the furan analogue **111** was improved in RLM when the ring was replaced with a pyridine to give analogue **112**. While the improvement in metabolic stability was modest, it eliminated the potential for bioactivation that might have occurred with the furan.

Six-Membered Heteroarenes. Six-membered heteroaryl groups are often used to replace aryl groups to improve the physical properties and metabolic stability of a drug.[9,21] However, six-membered heteroaryl groups may also be metabolized at either the carbon or heteroatom. Strategies for improving the metabolic stability of these rings often involve substituting or replacing the site of metabolism on the ring. Also, adding an additional nitrogen to the ring can serve to replace the site of metabolism, lower the $LogD_{7.4}$, and decrease the basicity of the ring. Table 3 summarizes some of the physical properties for common six-membered ring heteroarenes.

Six-Membered Heteroarenes. One Nitrogen. In their anticancer program on FMS-like receptor tyrosine kinase 3 (FLT3) inhibitors, Ishida showed how the metabolism of an analogue containing a pyridine was improved by blocking the site of metabolism or adding a nitrogen to the ring (Figure 33).[70] Metabolism studies on a related analogue suggested that

Table 3. cLogP, $cLogD_{7.4}$, and pK_a for Some Common Six-Membered Ring Heteroaromatics[c]

name	structure	cLogP[a]	$LogD_{7.4}$[a]	pK_a[b]
pyridine		0.645	0.72	5.2
pyridazine		-0.582	-0.77	2.3
pyrimidine		-0.312	-0.33	1.3
pyrazine		-0.312	-0.28	0.4

[a]See ref 24. [b]See ref 25. [c]pK_a refers to the Het ⇆ $HetH^+$ equilibrium.

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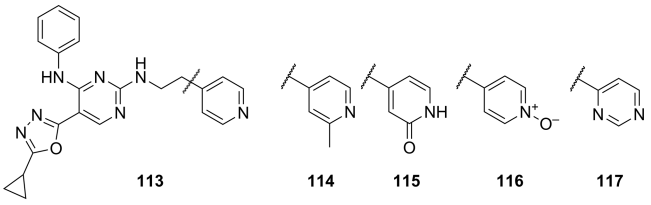
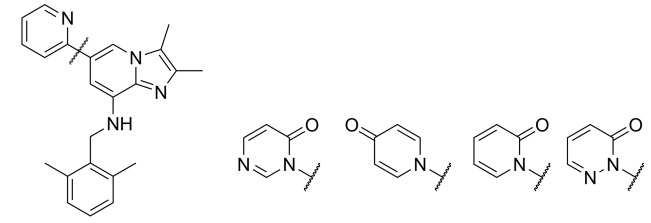


	113	114	115	116	117
MOLM-13 (GI_{50}) = 40		137	113	3610	775
HLM CL (h/L/kg) = 19		22	2.5	0.2	4.4
$clogD_{7.4}$ = 1.49		1.92	0.32	-0.42	0.44

Figure 33. In vitro potency and metabolic stability of selected FLT3 inhibitors[70] (MOLM-13 cells are from the human acute myeloid leukemia cell line).

the 2-position of the pyridine ring on analogue **113** was a major site of metabolism. To circumvent this problem, they blocked the 2-position with a methyl group (**114**), but it showed no improvement in metabolic stability. However, the 2-pyridone and pyridine *N*-oxide analogues **115** and **116**, respectively, had better metabolic stability than **113**. In addition, the analogue that contained a pyrimidine (**117**) had improved metabolic stability. Analogues **115**−**117** increased the polarity of the ring and decreased the electron density at the C-2 position as a means for improving metabolic stability.

Bailey and co-workers employed the strategy of reducing the polarity of the pyridine ring to improve the metabolic stability of their acid pump antagonist (APA) **118** (Figure 34).[71] The



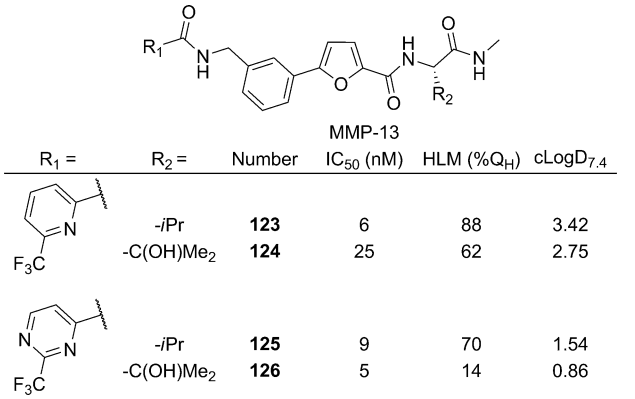
	118	119	120	121	122
H^+/K^+ ATPase pIC_{50} = 7.4		7.0	6.7	7.9	7.8
RLM CL_{int} (mL/min/g) = 13		2.5	2.5	6.0	9.6
$cLogD_{7.4}$ = 5.71		3.17	3.64	3.95	3.56

Figure 34. In vitro potency and metabolic stability for selected APAs.[71]

metabolic stability of **118** was improved by over 5-fold when the pyridine was replaced with the more polar pyrimidinone (**119**) or a pyridin-4-one (**120**) ring. Analogue **120** had the best overall profile with respect to potency and metabolic stability, so it was advanced into a rat acid secretion model where it was efficacious at 1 mg/kg, po.

The report by Gao et al. on matrix metalloproteinase 13 (MMP-13) inhibitors for rheumatoid and osteoarthritis provided another example where a pyrimidine analogue was more metabolically stable than its pyridine congener (Figure 35).[72] The pyrimidine analogue **125** was marginally more metabolically stable than the corresponding pyridine analogue **123**. However, for the analogues where R_1 = $C(OH)Me_2$, the metabolic stability of the pyrimidine analogue **126** was significantly better than the pyridine analogue **124**. The increased polarity that the pyrimidine ring provides may account for the improved metabolic stability of these analogues.

Another approach that has been used to improve the metabolic stability of compounds containing pyridines is to saturate the ring. In the Rho-associated coiled-coil containing

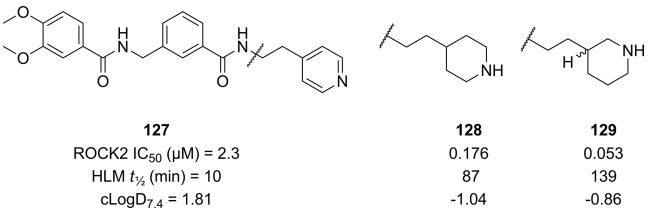


MMP-13

R_1 =	R_2 =	Number	IC_{50} (nM)	HLM (%Q_H)	$cLogD_{7.4}$
	-*i*Pr	**123**	6	88	3.42
	$-C(OH)Me_2$	**124**	25	62	2.75
	-*i*Pr	**125**	9	70	1.54
	$-C(OH)Me_2$	**126**	5	14	0.86

Figure 35. In vitro potency and metabolic stability of selected MMP-13 inhibitors.[72] %QH is the percent of hepatic clearance.

serine/threonine protein kinase type 2 (ROCK2) inhibitors reported by Morwick et al. their initial lead compound contained a pyridine (**127**), but it had poor metabolic stability (Figure 36).[73] Through SAR (structure−activity relationship)



	127	128	129
ROCK2 IC_{50} (μM) = 2.3		0.176	0.053
HLM $t_{½}$ (min) = 10		87	139
$cLogD_{7.4}$ = 1.81		-1.04	-0.86

Figure 36. In vitro potency and metabolic stability of selected ROCK2 inhibitors.[73]

studies, Morwick et al. found that the 4- and 3-piperidinyl analogues **128** and **129** had a significant increase in HLM stability compared to the parent pyridine analogue. The added polarity of the more basic piperidine rings may explain the improvement in metabolic stability.

An example of how metabolite identification studies can be used to design around metabolism issues on pyridines was illustrated by Ceccarelli and co-workers (Figure 37).[74] Compound **130** was a lead in their metabotropic glutamate receptor type 5 (mGlu5) antagonist program, but it was readily metabolized by microsomes. Metabolite identification studies revealed that the oxidation products on the upper ring were the major metabolites (analogues **131** and **132**), whereas metabolism on the lower ring was minor. Ceccarelli et al. prepared the two major metabolites and found that the added polarity of the hydroxyl groups improved the metabolic stability of these compounds, but the activity at mGlu5 suffered, so they focused on preparing analogues that were designed to block the sites of metabolism. Analogue **133** had a nitrogen at the site where metabolism was occurring on the upper ring of the parent compound, which led to an improvement in RLM. Compound **134** had the lower pyridine ring replaced with a pyrimidine, which also led to an improvement in RLM. They also removed the methyl group on the upper ring that was being metabolized and added a fluorine atom at another site of metabolism. This led to compound **135**, which was slightly less stable in RLM than parent compound **130**. However, by replacing the lower pyridine ring on **135** for a pyrimidine ring, they obtained analogue **136**, which had virtually no RLM

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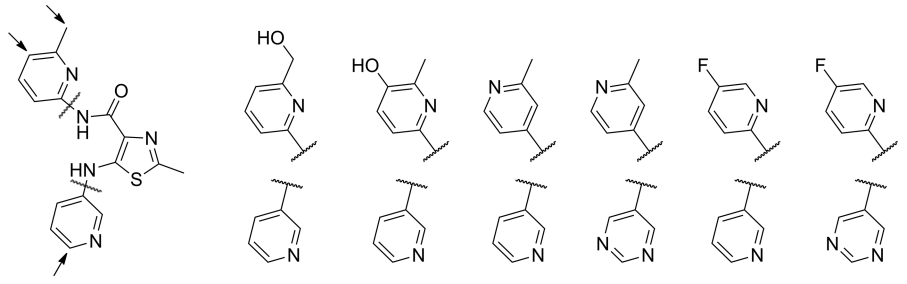

	130	131	132	133	134	135	136
mGlu5 K_i (nM) = 9 nM		67	111	44	81	13	31
RLM CL (μL/min/mg) = 135.8		88.9	75.2	78.9	25.3	188	0
$cLogD_{7.4}$ = 1.52		-0.12	1.40	2.35	1.81	1.50	0.95

Figure 37. In vitro potency and metabolic stability of selected mGlu5 antagonists.[74] Arrows indicate sites of oxidation observed on metabolites generated with HLM.

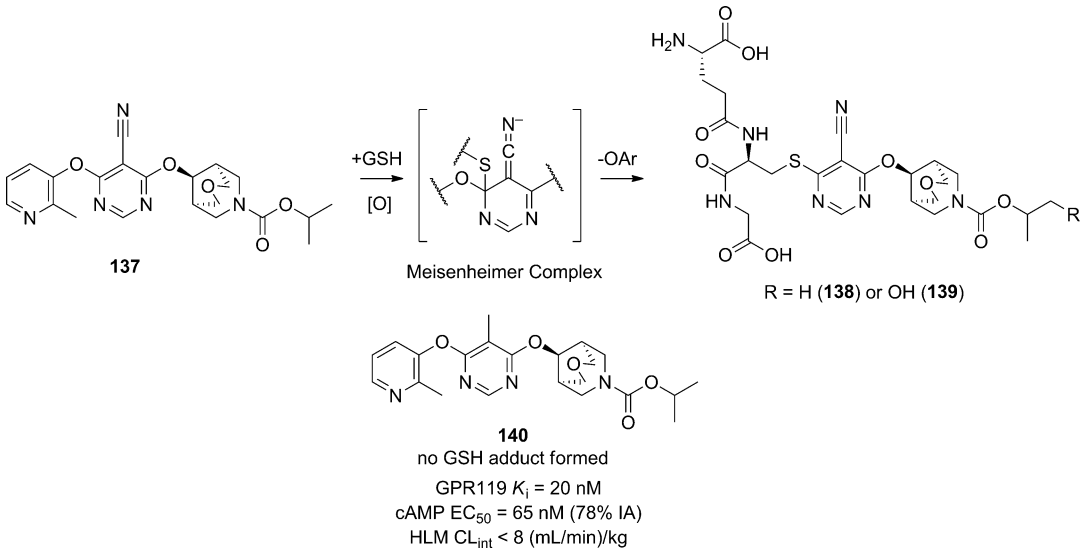

Figure 38. GPR119 agonists containing 5-substituted pyrimidines: propensity for GSH adduct formation.[75,76]

turnover and was orally active in their in vivo model for anxiety (MED = 3 mg/kg, po).

Six-Membered Heteroarenes: Two Nitrogens. While the strategy of adding a nitrogen to a pyridine ring can be a useful for reducing metabolism, there are examples when the heterocycle with two nitrogens has to be modified to improve metabolism. For example, in their anti-diabetes program on G-protein-coupled receptor 119 (GPR119) agonists, Kalgutkar et al. observed that the pyrimidine compound **137** underwent metabolic activation and GSH conjugation to give two major metabolites **138** and **139** (Figure 38).[75] The authors proposed that the GSH adducts formed through a nucleophilic aromatic substitution reaction onto the pyrimidine. The negatively charged Meisenheimer complex that forms in the proposed mechanism would be stabilized by the 5-cyano group on the pyrimidine ring. Kalgutkar et al. reasoned that if they added a group to the pyrimidine ring that destabilized the Meisenheimer complex, they should reduce the formation of GSH adducts. To that end, they prepared the 5-methylpyrimidine derivative **140** that, upon incubation with HLM, GSH, and NADPH, did not form GSH adducts. Presumably, the 5-methyl group destabilizes the buildup of charge on the pyrimidine that would be necessary for the Meisenheimer complex to form. While the biological activity of the 5-cyano analogue was not disclosed, the 5-methylpyrimidine was reported in another paper to be an important analogue for understanding the conformational preferences of GPR119 agonism.[76]

Another example of how GSH conjugation was reduced on a six-membered heterocycle containing two nitrogens was illustrated in a report on corticotropin-releasing factor-1 receptor (CRF_1) antagonists containing a pyrazin-2(1*H*)-one (Figure 39). Hartz et al. initially reported on lead compound **141**, which was orally efficacious in a rodent model of anxiety but displayed high rat iv clearance.[77] Metabolite identification studies indicated that the peripheral methoxy groups were the main sites of metabolism, and oxidation on the pyrazin-2(1*H*)-one was negligible. Further SAR studies on this series, directed at replacing or removing the metabolically labile methoxy groups, led them to BMS-665053[78] (**142**), a compound that had a 10-fold improvement in rat iv clearance.[79] Additional metabolite ID studies on **142** revealed that the pyrazin-2(1*H*)-one was susceptible to oxidation and GSH conjugation.[78] The suggested intermediate that led to pyrazin-2(1*H*)-one metabolism was the chloroepoxide **146**, which after hydrolysis or reaction with GSH led to the observed oxidized metabolites **149** and **150** and GSH adducts **147** and **148**. Metabolism was also observed on the arylamine portion of **142** but to a lesser extent. Replacing the chloride on the pyrazin-2(1*H*)-one ring with a bromide gave compound **143**, which in metabolism studies with RLM showed slightly less oxidation and GSH

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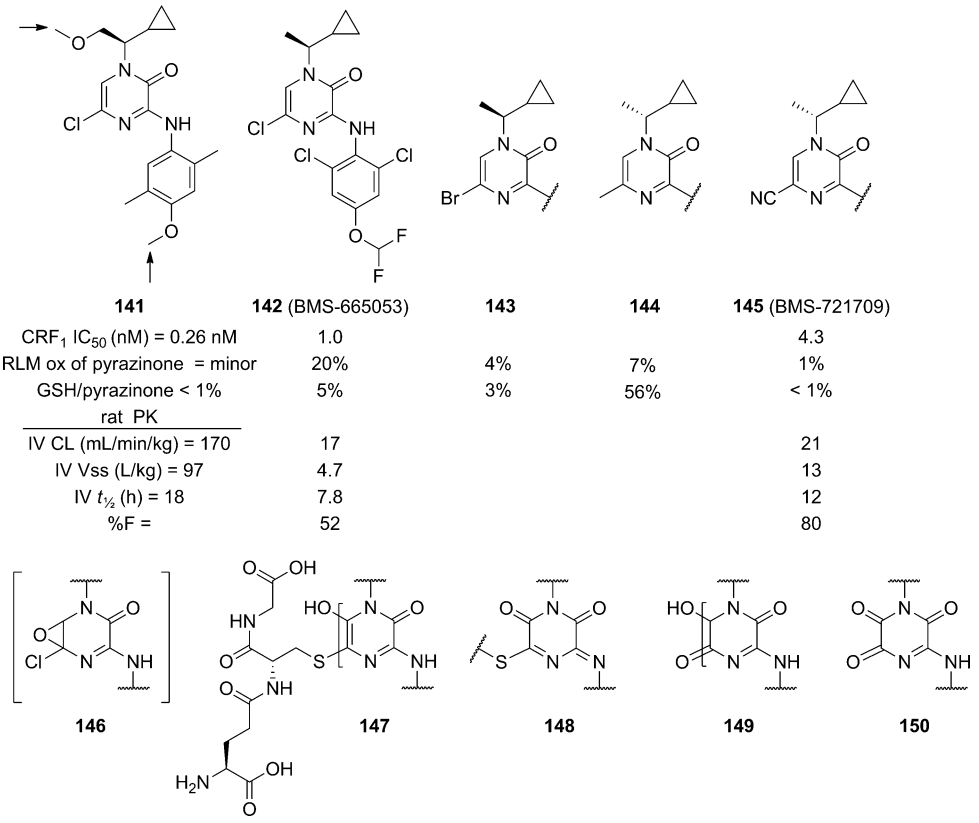

Figure 39. CRF_1 antagonists containing pyrazin-2(1*H*)-ones: propensity for ring oxidation and GSH adduct formation.[77−79] A single bracket on the structure of the metabolites indicates that the regiochemistry has not been assigned. Arrows indicate sites of metabolism.

conjugation. The 5-methyl analogue **144** showed a significant increase in GSH adduct formation on the pyrazin-2(1*H*)-one ring over the 5-chloro analogue, while the 5-cyano analogue **145** had negligible metabolism at the pyrazin-2(1*H*)-one ring. The trend in the amount of metabolism observed on the pyrazin-2(1*H*)-one ring can in part be explained by the inductive effects of the substituents at the 5-positon. The electron withdrawing 5-cyano group makes the double bond less prone to oxidation, while the electron rich 5-methyl group makes it more susceptible to oxidation. Bromide and chloride are considered to be less electron withdrawing than the cyano group,[80,81] which might explain why the 5-bromo and 5-chloro analogues show more metabolism on the pyrazin-2(1*H*)-one ring than **145**. Analogues **142** and **143** differ from **144** and **145** by the stereochemistry of the α-methyl group. How this subtle change influenced the metabolism cannot be determined, since analogues with the same α-methyl stereochemistry were not reported. Nevertheless, the trend in GSH conjugation of the pyrazinone ring for analogues **142**−**145** is consistent with the electronic withdrawing properties of the substituent on the 5-position. From the standpoint of potency, metabolism, and rat PK, the 5-cyano analogue (**145**, BMS-721709[78]) was the most promising lead in this report, but further development of this compound has not yet been disclosed.

■ FUSED BICYCLIC HETEROAROMATICS

As with the heteroaromatic rings discussed above, the strategies for reducing the metabolism of fused bicyclic heteroaromatics involve blocking the sites of metabolism and reducing the electron density of the ring by attaching polar groups or adding heteroatoms to the ring. For example, in their work on therapies for major depressive disorder, Bannwart et al. described the optimization of novel triple reuptake inhibitors (TRI) that contained a benzothiophene (**151**, Figure 40).[82]

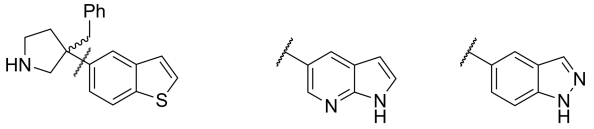

Figure 40. Monoamine reuptake K_i values and in vitro metabolism data for selected TRI.[82] SERT, NET, DAT are the serotonin transporter, norepinephrine transporter, and dopamine transporter, respectively.

The metabolic stability of analogue **151** was improved by replacing the ring with either an azaindole ring (**152**) or indazole ring (**153**). Both changes led to analogues that were less lipophilic than analogue **151**, which may explain the improved metabolic stability. In addition, if the benzothiophene ring on compound **151** is the site of metabolism, it may be prone to oxidation on the phenyl ring and/or sulfur atom, which is precedented in the literature.[13] Analogues **152** and **153** may have better stability than **151**, since the metabolically labile benzothiophene was replaced with an azaindole and indazole, respectively. While the indazole containing analogue **153** had the best metabolic stability in this set of compounds, it had the undesired attribute of being a CYP2D6 inhibitor.

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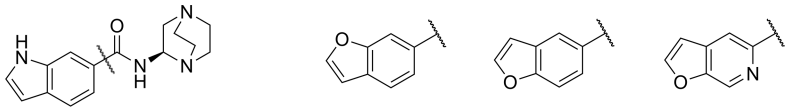
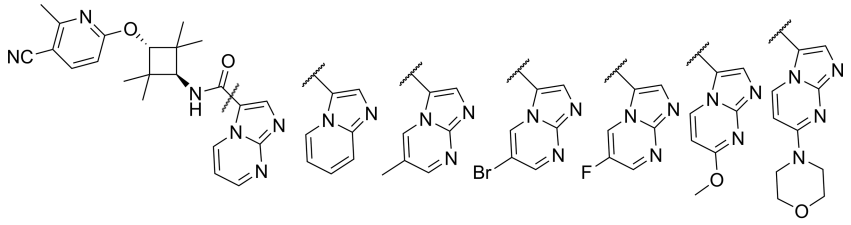


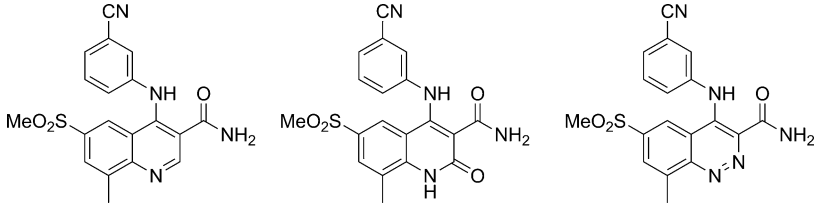
154	155	156	157
α7-5HT$_3$ chimera EC$_{50}$ (nM) = 100	86	47	65
RLM (% remaining) = 14	69	77	55
RMA = nd	RMA = Positive	RMA = Negative	RMA = Negative
cLogD$_{7.4}$ = 0.01	0.52	0.52	-0.52

Figure 41. In vitro activity and rat liver microsomal profiles for selected α7 nAChR agonists. RLM data are expressed as percent parent remaining after 1 h.[83] The reactive metabolite assay (RMA) was conducted in the presence of HLM and GSH-EE.



158	159	160	161	162	163	164
Metabolized by AO = Yes	No	Yes	Yes	Yes	No	No
cLogP = 4.51	5.51	5.01	5.38	4.66	5.38	4.64
HLM (ER) < 0.3	0.5					

Figure 42. SAR of selected AR antagonists toward oxidation by AO. A "yes" indicates that qualitative formation was observed in human S9 fraction (HS9) without NADPH which suggests AO oxidation. HLM is measured as extraction ratio (ER). cLogP values in this figure are those reported by Linton et al.[84]



165	166	167
hWB pIC$_{50}$ = 7.9	7.6	8.6
cLogD$_{7.4}$ = 3.09	-0.14	0.17
Cynomolgus Monkey PK		
%F = 33	52	64
CL$_p$ (mL/min/kg) = 82	5.9	1.6
$t_{½}$ (h) = 0.8	4.2	7.2

Figure 43. In vitro activity and in vivo PK parameters for selected PDE4 inhibitors.[87] The potency data are reported as the inhibition of LPS induced TNF-α production in human whole blood (hWB).

Another report containing examples of fused 5,6 heteroaromatic rings was published by Wishka et al. in their work on agonists for the α7 neuronal nicotinic acetylcholine receptor (α7 nAChR) (Figure 41).[83] The indole ring on analogue **154** was replaced with a benzofuran ring (**155**), which improved metabolic stability. However, benzofuran analogue **155** was positive in the reactive metabolite assay, indicating that an electrophilic metabolite was being formed. Additional metabolite identification studies with analogue **155** suggested that a glutathione ethyl ester (GSH-EE) adduct had formed on the phenyl ring of the benzofuran. The authors mentioned that the position of the carboxamide on the phenyl ring of **155** may have had an influence on bioactivation, since the regioisomeric benzofuran analogue **156**, which had a para-substituted carboxamide, was negative in the reactive metabolite assay. The related furopyridine analogue **157** was also negative in the reactive metabolite assay, but the added polarity of the pyridyl group did not improve the metabolic stability of the compound.

A paper containing the 5,6-heteroaromatic ring system, imidazopyrimidine, was published by Linton et al. from their androgen receptor (AR) antagonist program for prostate cancer (Figure 42).[84] Although the lead compound **158** had desirable rat PK, this compound was metabolized on the imidazopyrimidine ring by aldehyde oxidase (AO). Unlike CYP P450s, AO delivers an oxygen atom as a nucleophile and is known to add to electron-deficient carbons in N-heterocycles.[20,85,86] To decrease the amount of metabolism by AO, Linton et al. prepared an analogue with one less nitrogen in the ring to increase the electron density of the ring, which resulted in analogue **159** that was no longer a substrate for AO. While the oxidation through AO was minimized, CYP P450-mediated metabolism increased, which was thought to be due to the

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higher lipophilicity of **159**. To modify the electronics of the imidazopyrimidine ring, they also prepared analogues with various substituents at the 6-position (**160**−**162**). However, these compounds were all substrates of AO, even though their electronic properties and lipophilicity differed significantly. On the other hand, AO oxidation was eliminated when the 7-position was substituted with either a methoxy (**163**) or morpholine group (**164**), suggesting that this was the site of AO oxidation on compound **158**. This last example illustrates that, in some cases, making a N-heterocycle too electron deficient to avoid CYP P450 mediated oxidation may make it more prone to AO oxidation.

An example of a 6,6-heteroaromatic system was reported by Lunniss et al. in their phosphodiesterase 4 (PDE4) inhibitor program (Figure 43).[87] The compound containing a quinoline (**165**) had acceptable PK parameters in rat and dog, but they discovered that an oxidized analogue **166** was formed in monkey hepatocytes. This metabolite was synthetically prepared and was found to be more stable than the parent compound in monkey PK studies. To further improve the metabolic stability of compound **165**, the quinoline ring was replaced with a cinnoline to give analogue **167**. The added nitrogen in the cinnoline ring blocked the metabolic site of the quinoline and lowered the electron density of the ring.

■ CONCLUSIONS

In this review, we have given specific examples where the metabolic profile of the compound was improved by modifying the molecule at or near the heterocycle. In several of the examples discussed above, metabolite identification studies pointed to the heterocycle as the site of metabolism, and direct modification of the heterocycle led to an increase in metabolic stability. However, in the many of the other examples, the site of metabolism was not elucidated and may have occurred away from the heterocycle. So the effect that changing the heterocycle had on improving metabolism may have been indirect. Nonetheless, whether the change made to the heterocycle had a direct or indirect effect on metabolism, there is still value in examining the general trends used for improving the metabolism that emerge from this review.

For saturated heterocyclic rings, adding fluorine atoms, polar groups, or reducing the ring size helps to reduce metabolism. Fluorine atoms served to lower the electron density of the ring as well as block potential sites of metabolism. Polar groups were either appended to the ring or incorporated into the ring to give compounds with lower lipophilicity. In some cases adding a polar group had a detrimental effect on metabolism, as was illustrated by the example from Mastalerz et al., where the hydroxyl and lactam diazepane analogues were less metabolically stable than the unsubstituted diazepane derivative.[51] Reducing the ring size generally improved metabolic stability. However, there were some exceptions in examples with pyrrolidine rings where the larger piperidine analogue was found to be more metabolically stable.[31,32]

For five-membered heteroarenes, adding one or more heteroatoms usually results in an increase in polarity and a ring with lower electron density, which helps to reduce metabolism. For example, we found several examples where the more reactive thiophene, furan, and thiazole rings were replaced with less electron rich rings like isoxazoles, thiadiazole, oxadiazoles, pyrazoles, and triazoles that led to analogues with lower metabolism.

The position of the heteroatoms in five-membered heteroarenes also influences metabolism. In two examples,[65,68] analogues containing the 1,2,4-oxadiazole were less stable than their 1,3,4-oxadiazole congeners. A recent review comparing the HLM CL of 1,2,4- and 1,3,4-oxadiazole congeners confirms the fact that 1,3,4-oxadiazoles tend to be more metabolically stable.[88] Also, in the case of the triazole, there were two examples where the 1,2,4-triazole analogues were more stable than their 1,2,3-triazole congeners.[64,68]

Another method used to improve the metabolic profile of a five-membered heteroarene was to install a group that served as an alternative site for metabolism. This was illustrated in the work by Obach where they showed that on meloxicam the main site of metabolism was the methyl group which spared the thiazole ring from oxidative ring-opening that was seen with sudoxicam.[55] This was discovered retrospectively, but adding a decoy group to prevent the formation of reactive metabolites could be a useful strategy for preventing the bioactivation of other compounds containing a five-membered heteroarene.

For six-membered ring heteroarenes adding polar groups, fluorine atoms, or inserting nitrogens into the ring helps to decrease metabolism. Attaching electron withdrawing groups and adding nitrogen atoms to the ring generally had the beneficial effect of making these compounds less prone to metabolism. However, in some instances, an electron deficient ring was more prone to nucleophilic attack by GSH as demonstrated by Kalgutkar et al. in their work with the GPR119 agonist containing a 4-cyanopyrimidine.[75] Also, Linton et al. found that the electron deficient imidazopyrimidine, while relatively stable to CYPs, was susceptible to nucleophilic oxygen attack mediated by AO.[84]

Heterocycles are a broad class of compounds, covering a diverse set of ring systems with various types of heteroatoms. The heterocyclic compounds illustrated in this review cover only a small subset of this broad class of compounds, but the general principles of lowering lipophilicity and blocking sites of metabolism should serve as a useful starting point for solving metabolic issues on other heterocycles. Changing the ring size or the arrangement of the heteroatoms has also been used to improve metabolism, but the generality of these trends is less clear and their applicability to other heterocycles deserves further investigation. In the end, a certain amount of experimentation is necessary to understand the best way to improve the metabolism of any particular heterocycle. Knowing the identity of the metabolites can greatly facilitate this endeavor. The examples in this review should provide medicinal chemists with a foundation for understanding techniques used to solve the issues of heterocycle metabolism.

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■ ABBREVIATIONS USED

%QH, percent of hepatic clearance; %TO, percent turnover; 11β-HSD1, 11β-hydroxysteroid dehydrogenase type I; 5-HT1A, -1B, -2C, -1D, serotonin receptor subtypes 1A, 1B, 2C, and 1D; A_{2A} and A_{2B}, adenosine receptor types 1 and 2A; α7 nAChR, α7 neuronal nicotinic acetylcholine receptor; AO, aldehyde oxidase; APA, acid pump agonist; AR, androgen receptor; CB1 and CB2, cannabinoid receptors 1 and 2; CCR5, C−C chemokine receptor type 5; CL, clearance; CL_{int}, intrinsic clearance; COX, cyclooxygenase; CRF1, corticotropin-releasing factor-1 receptor; DAT, dopamine transporter; DLM, dog liver microsomes; DPP-4, dipeptidyl peptidase IV; EGFR, epidermal growth factor receptor; EphB3, erythropoietin-producing hepatocellular carcinoma receptor type B3; ER, extraction ratio; FLT3, FMS-like receptor tyrosine kinase 3; FXa, factor Xa; GPR119, G-protein-coupled receptor 119; GSH, glutathione; GSH-EE, glutathione ethyl ester; GlyT1, human glycine transporter 1; HCV, hepatitis C virus; HDAC1, histone deacetylase 1; HER2, human epidermal growth factor receptor 2; Hh, Hedgehog; HH3, histone H3; H_3R and H_4R, human histamine receptor subtypes 3 and 4; HLM, human liver microsomes; HS9, human S9 fraction; hWB, human whole blood; JAK2, Janus kinase 2; JNK, c-Jun N-terminal kinase; MF %, metabolic bioavailability prediction (assumes 100% total absorption); mGlu5, metabotropic glutamate receptor type 5; MLM, mouse liver microsomes; MMP-13, matrix metalloproteinase 13; MoLM, monkey liver microsomes; MOLM-13, human acute myeloid leukemia cell line; mTOR, mammalian target of rapamycin; MurI, glutamate racemase; NET, Norepinephrine transporter; PBMC, peripheral blood mononuclear cell; PDE4, phosphodiesterase 4; RLM, rat liver microsome; RMA, reactive metabolite assay; ROCK2, Rho-associated coiled-coil containing serine/threonine protein kinase type 2; SD, Sprague-Dawley rat; SERT, serotonin transporter; Tpo, thrombopoietin; TRI, triple reuptake inhibitor; UDPGA, uridine 5′-diphosphoglucuronic acid; V_{ss} and V_{dss}, volume of distribution, under steady state; V_z, volume of distribution

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