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[3-*cis*-3,5-Dimethyl-(1-piperazinyl)alkyl]-bis-(4′-fluorophenyl)amine Analogues as Novel Probes for the Dopamine Transporter

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Abstract—In a continuing effort to identify novel probes with which to study the dopamine transporter (DAT), we discovered that the σ receptor antagonist, rimcazole, binds with moderate affinity ($K_i = 224$ nM) to the DAT. The results from previous SAR studies suggested that substitution of the carbazole ring system of rimcazole with bis-(4′-fluorophenyl)amine might improve binding affinity and selectivity for the DAT. Thus, a novel series of [3-*cis*-3,5-dimethyl-(1-piperazinyl)alkyl]bis-(4′-fluorophenyl)amines were synthesized. The most potent compound in this series (**9b**) displaced [^{3}H]WIN 35,428 binding in rat caudate-putamen ($K_i = 17.6$ nM) with comparable affinity to GBR 12909. Despite high-affinity binding at DAT, and structural similarity to GBR 12909, preliminary studies suggest **9b** behaves more like rimcazole than GBR 12909 and does not demonstrate cocaine-like psychostimulant behavior in mice. © 2001 Published by Elsevier Science Ltd.

The psychomotor stimulant and reinforcing actions of cocaine are primarily mediated through the inhibition of dopamine reuptake and subsequent increases in synaptic dopamine levels.[1,2] The dopamine transporter (DAT) has thus provided a primary target for the development of medications with which to treat cocaine abuse and addiction.[3] However, the roles of the serotonin (SERT) and norepinephrine transporters (NET) in the reuptake of dopamine that have been identified in DAT knockout mice,[4,5] have lent support to investigating drugs that also have actions at these and other sites.[6,7] In addition, the modulation of cocaine's behavioral effects, and the attenuation of its toxic effects by drugs with activity at σ receptors[8–10] has suggested that agents that target this receptor system may have potential as treatment modalities for cocaine abuse and overdose.

Rimcazole (Chart 1), commonly classified as a σ antagonist and described as an atypical antipsychotic,[11] has been reported to attenuate the locomotor stimulatory effects produced by acute and subchronic administration of cocaine.[10,12] Although these effects were originally attributed to its σ receptor antagonism, the discovery that rimcazole had a 2-fold higher affinity for the DAT than σ receptors,[13] suggested that the DAT, may play a role in these actions.[14,15] For these reasons, we considered that rimcazole was an interesting lead compound for further drug development efforts.[14]

Although rimcazole bears no obvious structural similarity to cocaine, it does resemble a more potent and selective dopamine uptake inhibitor, GBR 12909.[16] GBR 12909 also binds with high affinity to σ receptors, and significant SAR efforts to identify structural requirements for both DAT and σ receptors have been reported.[17–22] Among the first series of rimcazole analogues, SH 3-24 ([3-(*cis*-3,5-dimethyl-4-[3-phenylpropyl]-1-piperazinyl)-propyl]diphenyl-amine), a compound that exhibited moderately high affinity for the DAT, ($K_i = 61$ nM) was identified.[14] SH 3-24 demonstrated equipotent binding affinity for σ_1 receptors ($K_i = 97$ nM) and was ~3-fold less potent at SERT ($K_i = 219$ nM).[14] However, like rimcazole, SH 3-24 did not exhibit cocaine-like behaviors in animal models of cocaine abuse.[23] In addition, SH 3-24, as well as its analogue SH 2-21 and rimcazole attenuated cocaine-induced convulsions.[15]

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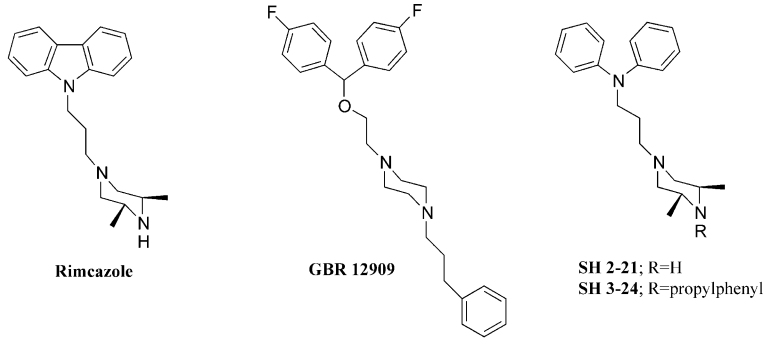
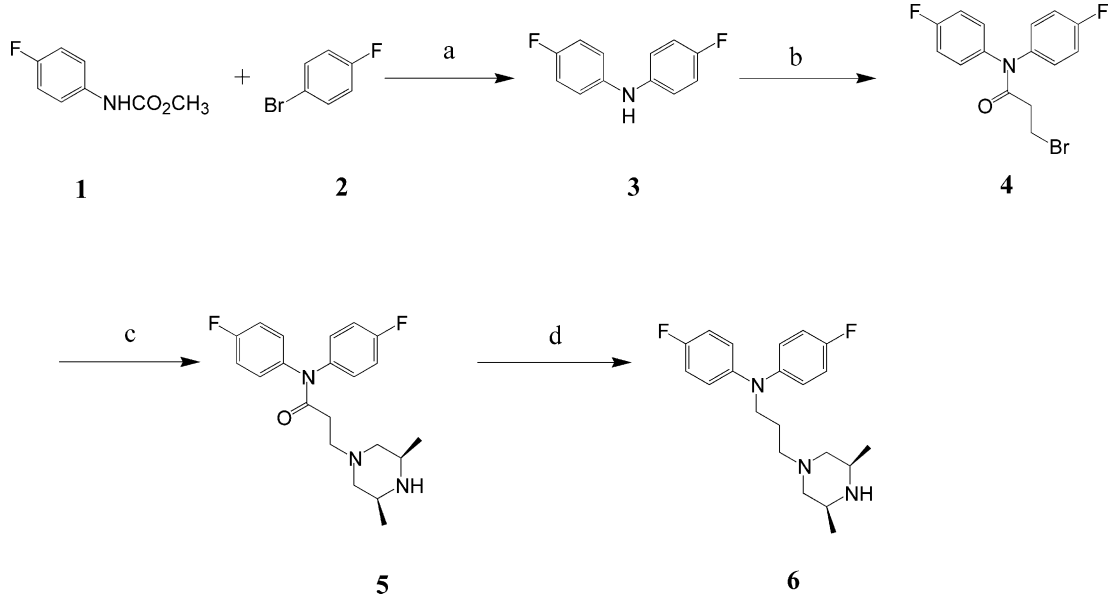


Chart 1.

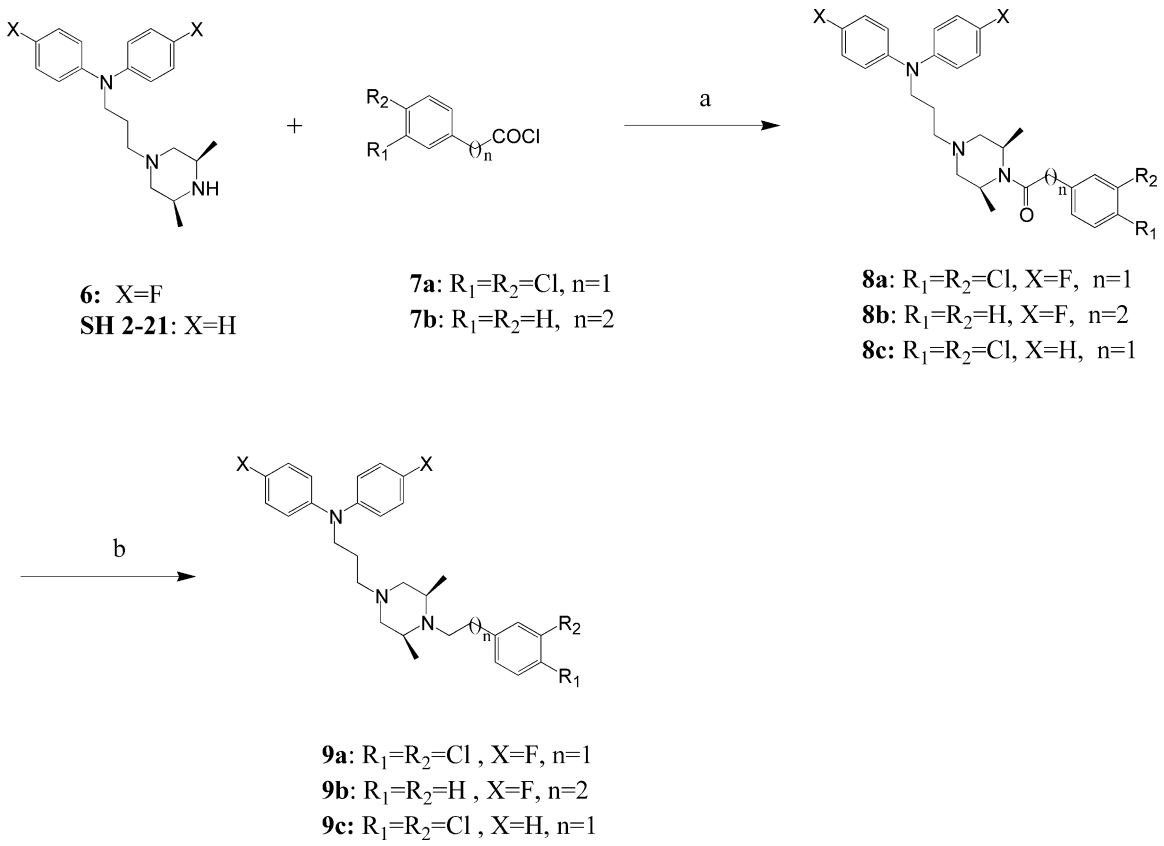
Based on the SAR that had previously been developed, the diphenylamine of SH 3-24 was substituted with *para*-F groups, and the terminal piperazine nitrogen was substituted with either propylphenyl or 3,4-dichlorophenethyl substituents. The latter group was chosen as it commonly appears in many of the most potent members of various other classes of DAT ligands and, in some cases, appears to be the driving force for binding at DAT.[24] These novel analogues were prepared and evaluated for binding at the DAT, SERT, and the NET.

The synthesis of novel analogues **6**, and **9a**–**c** are depicted in Schemes 1 and 2. In Scheme 1, the synthon, **3**, was prepared via a modification of the procedure of Cairi et al.[25] in 66% yield. Another procedure[26] was also attempted but the yield was significantly poorer (10%). Acylation of **3** with 3-bromopropanoylchloride gave **4** as a white solid[27] in 83% yield. Condensation of **4** with *cis*-2,6-dimethylpiperazine gave product **5**, in quantitative yield. Reduction with LAH gave the crude product **6**, which was purified by flash column chromatography (3% $CHCl_3$/MeOH/NH_4OH; 77% yield) and crystallized as the HCl salt.[28] In Scheme 2, compound **6** was reacted with the acid chloride **7a** or **7b**, prepared from the respective carboxylic acids, in refluxing $SOCl_2$, to give **8a** and **8b**, respectively. Compound **8c** was prepared in an analogous manner using SH 2-21 as the starting material. LAH reductions of the amide intermediates **8a**–**c** gave products **9a**–**c**, respectively, in 53–74% overall yield. All final products were purified by flash column chromatography (3% $CHCl_3$/MeOH/NH_4OH) and crystallized as their HCl or HBr salts.[29]

Binding affinities at DAT, SERT and NET, for the novel compounds **6**, **9a**–**9c**, are compared to rimcazole and GBR 12909, as well as SH 2-21 and SH 3-24, in Table 1. The methods for binding are described in full, elsewhere.[30] It is important to note, that [^{3}H]citalopram, rather than [^{3}H]paroxetine is used for labeling SERT and thus the K_i values for the reference compounds SH 2-21, SH 3-24 and rimcazole differ from those values previously reported.[14] Also, GBR 12909 was tested under the same assay conditions as rimcazole and its analogues so as to ensure comparability. For the DAT, substitution of the diaryl ring system with the bis-4′-

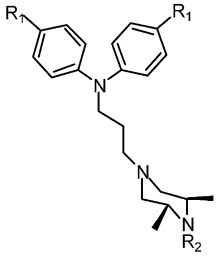


Scheme 1. Reagents and conditions: (a) (1) K_2CO_3, CuI, reflux, 10 h; (2) ethanol, KOH, reflux, 1.5 h; (b) 3-bromopropanoyl chloride, benzene, reflux, 5 h; (c) 2,6-dimethylpiperazine, DMF, H_2O, K_2CO_3, 80 °C 2 h; (d) LAH, THF, reflux, 2 h.



Scheme 2. Reagents and conditions: (a) toluene, reflux overnight; (b) LAH, THF, reflux, 2 h.

Table 1. Binding results at the monoamine transporters



Compd	R_1, R_2	K_i, nM ± SEM[a]			DAT/SERT	DAT/NET
		[^{3}H]WIN 35,428 (DAT)	[^{3}H]Citalopram (SERT)	[^{3}H]Nisoxetine (NET)		
SH 2-21	H, H	813 ± 22[b]	70,900 ± 6600	6810 ± 600[b]	87	8
SH 3-24	H, propylphenyl	61.0 ± 6.1[b]	1220 ± 160	3640 ± 440[b]	20	60
6	F, H	83.2 ± 8.3	13,100 ± 1800	16,900 ± 990	158	203
9a	F, 3,4-diCl-phenethyl	131 ± 17	4300 ± 260	> 100,000	33	> 760
9b	F, propylphenyl	17.6 ± 2.1	2130 ± 160	1020 ± 140	121	60
9c	H, 3,4-diCl-phenethyl	261 ± 60	5010 ± 580	> 30,000	19	> 115
rimcazole	—	224 ± 16	1710 ± 72	2160 ± 300[b]	8	10
GBR 12909	—	11.9 ± 1.9	105 ± 11	1270 ± 190	9	106

[a]Each K_i value represents data from at least three independent experiments, each performed in triplicate.
[b]Data from ref 14.

fluoro groups uniformly improved binding affinity. For the unsubstituted terminal piperazine analogue, **6**, binding affinity improved 10-fold from 813 nM for SH 2-21 to 83 nM. The combination of the 4′-bis-F and the terminal piperazine *N*-propylphenyl substitutions, resulted in the most potent compound in the series, **9b**, with a $K_i = 17.6$ nM, an affinity that is nearly equipotent to GBR 12909 ($K_i = 11.9$ nM). Interestingly, the 3,4-dichlorophenylethyl substituent (**9a,c**) did not yield high affinity ligands (**9a**; $K_i = 131$ nM, **9c**; $K_i = 261$ nM). This finding suggests that the 3,4-dichlorophenyl group is not sufficient to impart high-affinity binding at DAT,

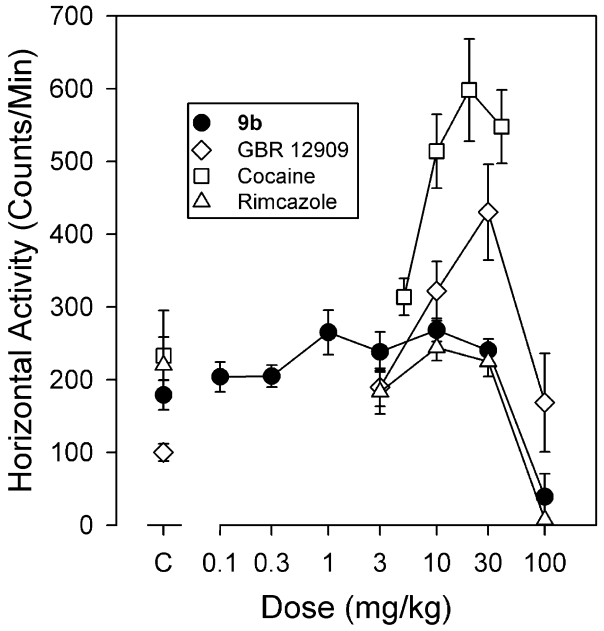


Figure 1. Dose-dependent effects of **9b** and several reference compounds on locomotor activity in mice. Ordinates: Rate of horizontal activity during a 30-min period after drug administration. Abscissae: dose of drug in mg/kg, log scale. Each point represents the average effect determined in eight mice. The effects of **9b** and cocaine were recorded during the first 30 min after injection to correspond with their periods of maximal stimulant activity. The effects of GBR 12909 and rimcazole were obtained between 30 and 60 min, and 20–50 min after injection, respectively, to correspond their slower onsets of action. Naive male Swiss–Webster mice (Taconic Farms, Germantown, NY) weighing 30–35 g, were placed singly in 40 cm^3 clear acrylic chambers which were contained in Digiscan activity monitors (Omnitech Electronics, Colombus, OH). Photoelectric sensors located 2.56 cm apart along the walls of the monitor counted horizontal activity. There were eight subjects per dose, and each animal was used only once. Compound **9b** produced only a marginal stimulation of locomotor activity that was statistically significant at 1.0 and 10.0 mg/kg. Note that **9b** had marginal efficacy as a locomotor stimulant compared to the effects of cocaine and GBR 12909.

which has also been observed in a similar series of compounds.[20]

Although the *N*-substituted analogues **9a–c** demonstrated an increase in SERT binding affinity as compared to the unsubstituted analogues SH 2-21 and **6**, none of the compounds demonstrated high affinity at SERT, and all were at least 10-fold less potent than GBR 12909. This resulted in significant DAT selectivity, as compared to GBR 12909, with both **6** and **9b** demonstrating >100-fold DAT/SERT selectivity. Likewise, none of the compounds demonstrated high affinity for NET, resulting in comparable or superior DAT/NET selectivity, as compared to GBR 12909 and rimcazole.

The effects of the most potent compound, **9b**, on locomotor behavior are shown in Figure 1. The methods for obtaining these data are described in full, elsewhere.[31] As has been demonstrated,[32] both cocaine and GBR 12909 produced a dose-related stimulation of locomotor activity, which is a hallmark effect of psychomotor stimulant drugs, and in particular indirect dopaminergic agonists.[33] The effects of cocaine (recorded for the first 30 min after injection to correspond with its rapid onset of action and relatively short half-life), were greater than those of GBR 12909 (recorded between 30 and 60 min after injection to correspond with its slower onset of action and relatively long half-life). At the highest doses each of these compounds increased activity less than what was obtained at a lower dose, a result typically obtained with psychomotor stimulant drugs.[33] Despite a DAT affinity comparable to that of GBR 12909, **9b** produced only a weak stimulation of locomotor activity, in the first 30 min after injection; the maximum stimulation was approximately 250 counts per min at 1.0 mg/kg, compared to the approximate 425 counts per min produced by 30 mg/kg of GBR 12909. In the second 30 min after injection, **9b** did not significantly stimulate activity (data not shown). Thus over the range of doses from those having no observable effects through doses that produced a profound disruption and eventual elimination of locomotor activity **9b** was relatively inactive as a locomotor stimulant drug.

Thus, the novel compound **9b** shows an increased affinity for the DAT compared to the parent compound, rimcazole that is comparable to that of the related piperazine, GBR 12909. In addition, this compound was selective for the DAT over the other monoamine transporters. This binding profile, however, did not confer locomotor behavioral effects, but rather **9b** lacked stimulant effects typical of drugs that act by interfering with dopamine uptake. Evaluation of σ receptor binding is underway as is the design and synthesis of novel analogues that have combinations of activities at the monoamine transporter systems. Obviously additional studies are required to characterize the pharmacology of these compounds, however, those presented here suggest that **9b** is representative of a class of dopamine uptake inhibitors that do not produce the typical profile of psychomotor stimulant effects.

Acknowledgements

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References and Notes

1. Wise, R. A. *Annu. Rev. Neurosci.* **1996**, *19*, 319.
2. Kuhar, M. J.; Ritz, M. C.; Boja, J. W. *Trends Neurosci.* **1991**, *14*, 29.
3. Carroll, F. I.; Howell, L. I.; Kuhar, M. J. *J. Med. Chem.* **1999**, *42*, 2721.
4. Carboni, E.; Speilewoy, C.; Vacca, C.; Nosten-Bertrand, M.; Giros, B.; DiChiara, G. *J. Neurosci.* **2001**, *21*, RC141.
5. Sora, I.; Hall, F. S.; Andrews, A. M.; Itokowa, M.; Li, X.-F.; Wei, H.-B.; Wichems, C.; Lesch, K.-P.; Murphy, D. L.; Uhl, G. R. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5300.
6. Gu, X.-H.; Yu, H.; Jacobson, A. E.; Rothman, R. B.; Dersch, C. M.; George, C.; Flippen-Anderson, J. L.; Rice, K. C. *J. Med. Chem.* **2000**, *43*, 4868.

7. Tamiz, A. P.; Zhang, J.; Flippen-Anderson, J. L.; Zhang, M.; Johnson, K. M.; Deschaux, O.; Tella, S.; Kozikowski, A. P. *J. Med. Chem.* **2000**, *43*, 1215.
8. McCracken, K. A.; Bowen, W. D.; deCosta, B. R.; Matsumoto, R. R. *Eur. J. Pharmacol.* **1999**, *370*, 225.
9. McCracken, K. A.; Bowen, W. D.; Matsumoto, R. R. *Eur. J. Pharmacol.* **1999**, *365*, 35.
10. Menkel, M.; Terry, P.; Katz, J. L.; Witkin, J. M. *Eur. J. Pharmacol.* **1991**, *201*, 251.
11. Ferris, R. M.; Tang, F. L. M.; Chang, K.-J.; Russell, A. *Life Sci.* **1986**, *38*, 2329.
12. Ujike, H.; Kuroda, S.; Otsuki, S. *Eur. J. Pharmacol.* **1996**, *296*, 123.
13. Izenwasser, S.; Newman, A. H.; Katz, J. L. *Eur. J. Pharmacol.* **1993**, *243*, 201.
14. Husbands, S. M.; Izenwasser, S.; Kopajtic, T.; Bowen, W. D.; Vilner, B. J.; Katz, J. L.; Newman, A. H. *J. Med. Chem.* **1999**, *42*, 4446.
15. Matsumoto, R. R.; Hewett, K. L.; Pouw, B.; Bowen, W. D.; Husbands, S. M.; Cao, J.; Newman, A. H. *Neuropharmacol.* **2001** in press.
16. Van der Zee, P.; Koger, H. S.; Gootjes, J.; Hespe, W. *Eur. J. Med. Chem.* **1980**, *15*, 363.
17. Matecka, D. M.; Lewis, D.; Rothman, R. B.; Dersch, C. M.; Wojnicki, F. H. E.; Glowa, J. R.; Devries, A. C.; Pert, A.; Rice, K. C. *J. Med. Chem.* **1997**, *40*, 705.
18. Matecka, D.; Rothman, R. B.; Radesca, L.; deCosta, B. R.; Dersch, C. M.; Partilla, J. S.; Pert, A.; Glowa, J. R.; Wojnicki, F. H. E.; Rice, K. C. *J. Med. Chem.* **1996**, *39*, 4704.
19. Dutta, A. K.; Xu, C.; Reith, M. E. A. *J. Med. Chem.* **1996**, *39*, 749.
20. Dutta, A. K.; Coffey, L. L.; Reith, M. E. A. *J. Med. Chem.* **1997**, *40*, 35.
21. Dutta, A. K.; Coffey, L. L.; Reith, M. E. A. *J. Med. Chem.* **1998**, *41*, 699.
22. Choi, S.-W.; Elmaleh, D. R.; Hanson, R. N.; Fischman, A. J. *J. Med. Chem.* **2000**, *43*, 205.
23. Katz, J. L.; Libby, A.; Husbands, S. M.; Newman, A. H. In *NIDA Research Monograph, 180*, Harris, L. S., Ed.; U.S. Department of Health and Human Services, NIH, Washington, DC. 2000; p 254.
24. Meltzer, P. C.; Blundell, P.; Yong, Y. F.; Chen, Z.; George, C.; Gonzalez, M. D.; Madras, B. K. *J. Med. Chem.* **2000**, *43*, 2982.
25. Cairi, M.; Gerig, J. T. *J. Am. Chem. Soc.* **1983**, *105*, 4793.
26. Wise, L. D.; Pattison, I. C.; Butler, D. E.; DeWald, H. A.; Lewis, E. P.; Lobbestael, S. J.; Nordin, I. C.; Poschel, B. P. H.; Coughenour, L. L. *J. Med. Chem.* **1985**, *28*, 606.
27. Note 1. Compound **4**: mp 96–97 °C, IR ($CHCl_3$) 1660 (CO) cm^{-1}, ^1H NMR δ 2.82 (2H, t, $J=6.63$ Hz), 3.65 (2H, t, $J=6.63$ Hz), 6.92–7.26 (8H, m, aryl–Hs), MS(EI) 339 (M+).
28. Note 2. Compound **6**: mp 234–236 °C (dec), ^1H NMR δ 1.04 (6H, d, 2 CH_3), 1.55 (2H, t, $J=10.5$ Hz), 1.78 (2H, t, $J=7.02$ Hz), 2.33 (2H, t, $J=7.02$ Hz), 2.72–2.92 (4H, m), 3.66 (2H, t, $J=7.14$ Hz, $CH_2N(PhF)_2$), 6.92–6.95 (8H, m, aryl-Hs), ^{13}C NMR δ 20.3, 25.0, 50.9, 51.1, 56.0, 61.1, 116.1, 116.4, 122.6, 122.7, 145.0, 156.7, 159.8, 168.1, MS(EI) 359 (M+), Anal. ($C_{21}H_{27}F_2N_3 \cdot 2HCl$), C, H, N.
29. Note 3. Compound **9a**: mp 211–213 °C (dec) ^1H NMR δ 1.12 (6H, d, $J=6.00$ Hz, 2 CH_3), 1.61–1.85 (4H, m), 2.31 (2H, t, $J=7.13$ Hz), 2.61–2.77 (6H, m), 2.92–2.98 (2H, m), 3.66 (2H, t, $J=7.22$ Hz, $CH_2N(PhF)_2$), 6.88–7.00 (7H, m, aryl–Hs), 7.25 (2H, d, $J=9.06$ Hz, aryl-Hs), 7.35 (2H, d, $J=8.16$ Hz, aryl–Hs), ^{13}C NMR δ 18.1, 24.7, 29.1, 49.8, 50.7, 53.5, 55.3, 61.3, 115.8, 116.1, 122.3, 122.4, 128.0, 130.4, 130.5, 144.6, MS (EI) 531 (M+), 218 ($^+CH_2N(PhF)_2$), Anal. ($C_{29}H_{33}F_2N_3Cl_2 \cdot 2HCl \cdot H_2O$), C, H, N. **9b**: mp 229.5–230.5 °C, ^1H NMR δ 0.98 (6H, d, $J=5.85$ Hz, 2 CH_3), 1.22–1.25 (2H, m), 1.58–1.80 (4H, m), 2.25–2.29 (2H, m), 2.51–2.54 (2H, m), 2.56–2.68 (4H, m), 2.78 (2H, t, $J=7.89$ Hz, CH_2Ph), 3.64 (2H, t, $J=7.08$ Hz, $CH_2N(PhF)_2$), 6.92–7.36 (13H, m, aryl–Hs), ^{13}C NMR δ 18.0, 24.6, 33.8, 47.5, 50.7, 53.5, 55.3, 61.4, 115.7, 116.0, 122.2, 122.3, 125.8, 128.3, 128.4, MS (EI) 477 (M+), 218 ($^+CH_2N(PhF)_2$), Anal. ($C_{30}H_{37}F_2N_3 \cdot 2HBr$), C, H, N. **9c**: mp 221–224 °C, ^1H NMR δ 1.11 (6H, d, $J=6.09$ Hz, 2×CH_3), 1.64–1.88 (4H, m), 2.32 (2H, t, $J=7.23$ Hz), 2.61–2.78 (6H, m), 2.92–2.98 (2H, m), 3.75 (2H, t, $J=7.29$ Hz, $CH_2N(PhF)_2$), 6.91–7.36 (13H, m, aryl–Hs), ^{13}C NMR δ 18.0, 24.5, 28.8, 49.6, 50.0, 53.3, 55.2, 61.2, 120.8, 121.0, 127.9, 129.1, 130.3, 130.4, 140.8, 147.9, MS (EI) 495 (M+), Anal. ($C_{29}H_{35}N_3Cl_2 \cdot 2HCl \cdot 0.5H_2O$), C, H, N.
30. Newman, A. H.; Robarge, M. J.; Howard, I. M.; Wittkopp, S. L.; Kopajtic, T.; Izenwasser, S.; Katz, J. L. *J. Med. Chem.* **2001**, *44*, 633.
31. Katz, J. L.; Izenwasser, S.; Kline, R. H.; Allen, A. C.; Newman, A. H. *J. Pharmacol. Exp. Ther.* **2000**, *288*, 302.
32. Izenwasser, S.; Terry, P.; Heller, B.; Witkin, J. M.; Katz, J. L. *Eur. J. Pharmacol.* **1994**, *263*, 277.
33. Kelleher, R. T. Psychomotor Stimulants: The Clinical and Basic Aspects. In *Drug Abuse*; Pradhan, S. N., Dutta, S. N., Eds.; C. V. Mosby Company; St. Louis, MO 1977; p 116.