

# Structure-Activity Relationships of Synthetic Cathinones

Richard A. Glennon and Małgorzata Dukat

**Abstract** Until recently, there was rather little interest in the structure-activity relationships (SARs) of cathinone analogs because so few agents were available and because they represented a relatively minor drug abuse problem. Most of the early SAR was formulated on the basis of behavioral (e.g., locomotor and drug discrimination) studies using rodents. With the emergence on the clandestine market in the last few years of a large number of new cathinone analogs, termed “synthetic cathinones”, and the realization that they likely act at dopamine, norepinephrine, and/or serotonin transporters as releasing agents (i.e., as substrates) or reuptake inhibitors (i.e., as transport blockers), it has now become possible to better examine their SAR and even their quantitative SAR (QSAR), in a more effective and systematic manner. An SAR picture is beginning to emerge, and key structural features, such as the nature of the terminal amine, the size of the  $\alpha$ -substituent, stereochemistry, and the presence and position of aromatic substituents, are being found to impact action (i.e., as releasing agents or reuptake inhibitors) and transporter selectivity.

**Keywords** DAT • Methcathinone • Monoamine transporters • NET • QSAR • SAR • SERT

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## 1 Introduction

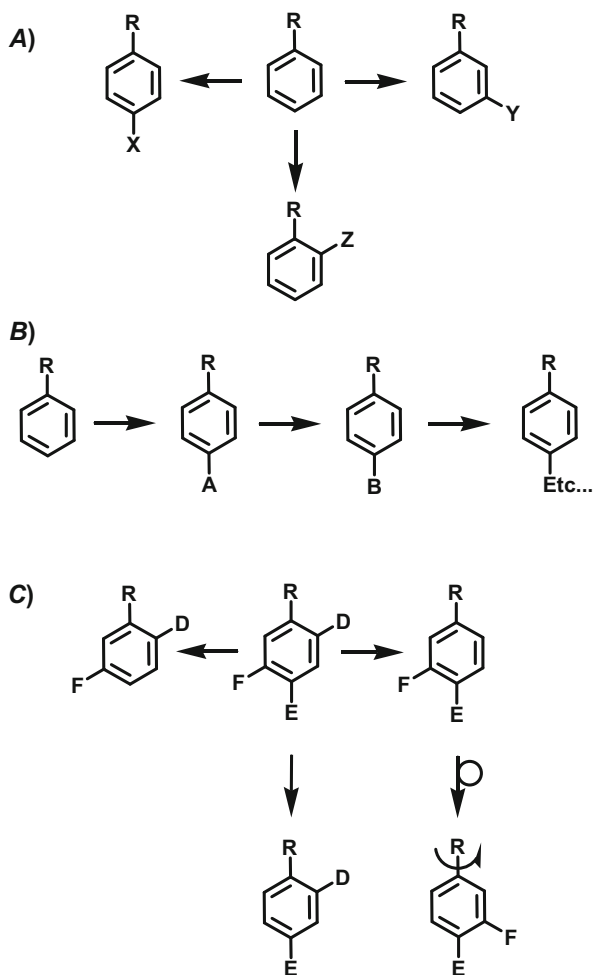
*Structure-activity relationship (SAR)* studies aim to define the qualitative influence of chemical structure on a given biological action and are focused on identifying *what*, and to what extent, substituents and – where applicable – the stereochemistry of substituents alters activity (i.e., action, potency). Recognized, but not widely acknowledged, is that more than a single SAR might be formulated for a given series of agents [1]. Consider that the behavioral actions of a series of agents might be related to their ability to activate a specific neurotransmitter receptor in the brain. An SAR might be formulated for these agents to bind at the target receptor using an in vitro radioligand binding assay (often referred to as a *structure-affinity relationship* or *SAFIR* study), whereas a different SAR might be formulated for their ability to act as agonists in an in vitro functional assay (e.g., some of the agents that display affinity for the receptor might function as weak partial agonists or antagonists rather than as agonists at the receptor of interest). Additionally, compounds that fail to bind at the receptor might act in an *allosteric* manner. Furthermore, the SARs for these actions might differ from SAR derived from their behavioral actions because some of the agents might be rapidly metabolized in vivo, or might be unable to penetrate the blood-brain barrier to reach their intended target. SAR is essentially linked to the assay from which the biological data were obtained, and the formulated SAR is not always conveniently extrapolated to different pharmacological actions/assays.

*Quantitative structure-activity relationship (QSAR)* studies attempt to explain *why/how* certain structural features influence the actions of a given series of agents. Once SAR studies have been conducted, a QSAR study can be performed using correlational analysis (often referred to as a *Hansch analysis*) to identify whether action/potency within a series of agents might be related to a specific physicochemical property of the substituent being altered. Measures include, but are not limited to, electronic character (as measured by the Taft steric parameter  $E_s$  or Hammett  $\sigma$  value), steric size (e.g., volume), overall or specific shape (e.g., Verloop parameters), and lipophilicity ( $\pi$  values). Other parameters consider the molecule as a whole.

Typically, SAR and QSAR studies are not an end unto themselves; rather, they are a means to an end. For example, the results of such studies can be employed (1) in drug design, to enhance the potency or selectivity of an agent; to reduce side (or off-target) effects; to reduce toxicity, or to alter metabolism; and (2) to investigate mechanisms of drug action.

As a general caveat, SAR and QSAR studies should focus on data derived from a common assay – ideally, data generated from the same laboratory and obtained

**Fig. 1** Examples of three types of SAR studies that can be pursued. Panel (A) depicts a nonlinear SAR study where the structure of a molecule is modified one substituent at a time; results can be related back to a common molecule in a systematic manner. Panel (B) exemplifies how structures can be related to one another by a single and, usually, position-consistent, structural alteration. Panel (C) is a combination of these two approaches and depicts the concept of “deconstruction”



under similar conditions. Employing biological data for one compound from one study with data from (an)other compound(s) from different laboratories or from unrelated studies does not provide reliable SAR results other than from, perhaps, a simple qualitative perspective. The latter approach is not uncommon when a new agent has been identified and attempts are being made to learn something of interest. Certainly, such data are not amenable to QSAR studies. Better yet are data generated where the structure of a molecule is modified one substituent at a time whereby results can be related back to a common molecule in a systematic manner (i.e., nonlinear SAR; see Fig. 1A), or where structures can be related to one another by a single structural alteration (linear SAR; see Fig. 1B). Another approach to SAR is to “deconstruct” a molecule by removing one substituent at a time to identify its influence on a particular action (see Fig. 1C); the latter is actually a combination of the above two approaches.

All of the above approaches are commonly used to formulate SAR, and, indeed, most have been employed to investigate the SAR of synthetic cathinones. However, it should be realized that results emanating from these SAR studies are not always unambiguous. For example, considering the “deconstruction” approach, substituents E and F (Fig. 1C) might influence one another. That is, the presence of substituent E (or F) might be impacted by the adjacent substituent (i.e., one of these substituents might alter the orientation or role of the other – e.g., E might hydrogen bond with F, but in the absence of F it cannot – or E and F might sterically repel one another, changing their steric orientation, which would not be the case when one of the two substituents has been removed). The possibility of rotameric binding also exists. For example, when the E and F substituents are present along with the D substituent, binding might occur in a manner dictated by the D substituent. However, when the D substituent is absent, rotameric binding might occur. That is, the E/F (only)-substituted compound might bind as shown in the lower right hand corner of Fig. 1C. As a consequence, SAR studies, certainly “deconstruction” studies, must take these possibilities to the heart, and SAR should be formulated conservatively; alternate SAR interpretations could be possible.

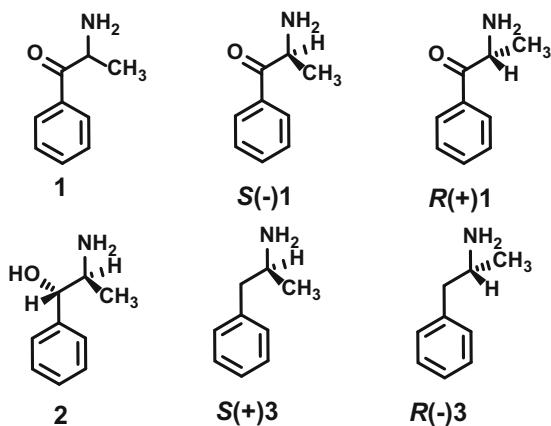
This chapter attempts to capture the SAR and, where possible, to examine the QSAR, of the synthetic cathinones. Unfortunately, due to the variety of assays employed over the last several decades, strict SAR comparisons are often difficult to make, and most studies of synthetic cathinones have been typically of an *agent-only* nature. That is, most investigators, and rightfully so, have tended to focus on individual agents appearing on the clandestine market in order to characterize and classify them. Sometimes, several agents might have been examined in the same study, but, because the agents possessed multiple structural alterations, it can be difficult, if not impossible, to formulate reliable SAR. However, the number of new synthetic cathinones is increasing at an alarming rate. In a United Nations Office of Drugs and Crime report [2], 44 synthetic cathinones had already been identified as having appeared on the clandestine market prior to 2013. According to a European Drug Report, there were greater than 10,000 seizures of synthetic cathinones in Europe in 2013 alone, and 70 were identified as being “new” analogs that had not been encountered hitherto [3]. In 2014, of 101 new psychoactive substances not previously identified, synthetic cathinones represented the largest single category (31%). And new synthetic cathinones continue to appear. Hence, although an agent-by-agent investigation would be required to fully characterize the pharmacological actions of these 150 or so entities, SAR and QSAR studies on a more limited number of agents might assist in providing tentative classifications and guidance as to what might be expected in terms of action(s) and potency. One intent of SAR/QSAR studies with synthetic cathinones is to forecast the actions of agents that have yet to appear (or that have recently appeared) on the clandestine market. It is not the intent of this chapter to review the overall pharmacology of synthetic cathinones; rather, it is to examine SAR. For the most part, only those studies addressing SAR, or studies where some SAR can be formulated in a retrospective manner, will be cited. The information will be provided in, more or less, chronological order so that the reader can appreciate some of the problems that were

encountered along the way. The remainder of this chapter is divided into three sections. The first section deals with early SAR studies involving cathinone culminating in the identification of methcathinone; the second section focusses on initial SAR investigations involving methcathinone, and the final section describes the most recent SAR and some QSAR findings.

## 2 Early SAR of Cathinone

These studies represent those conducted between the time of discovery of cathinone (**1**) and progress into the mid- to late 1990s. Cathinone, specifically *S*(-)-cathinone or *S*(-)-**1** (see Fig. 2 for chemical structures) was identified in 1975 as the active stimulant component of the shrub *Catha edulis*. Upon its discovery, *S*(-)-cathinone (*S*(-)-**1**) was simply referred to as “cathinone”; *note*: early studies used the term “cathinone” to refer only to the *S*(-)-isomer, whereas more recent studies use the term “cathinone” to refer to racemic or ( $\pm$ )-cathinone unless stereochemistry is specifically defined. Prior to 1975, it was thought that (+)-cathine (**2**, Fig. 2) represented the major stimulant constituent of the plant. Not unexpectedly, then, some of the first investigations focused on a pharmacological comparison of these two agents, cathinone and (+)-cathine, and on the stereochemical aspects of cathinone. The United Nations Narcotics Laboratory synthesized cathinone and its optical isomers in 1978 and made samples available shortly thereafter. It was soon shown that cathinone (**1**) was more potent than (+)-cathine (**2**) as a locomotor stimulant in rodents and in other behavioral studies (reviewed: [4]). For example, in tests of stimulus generalization using rats trained to discriminate *S*(+)-amphetamine (AMPH, *S*(+)-**3**, Fig. 2), *S*(-)-cathinone (*S*(-)-**1**) was several times more potent than (+)-cathine (**2**), and nearly as potent, typically more potent, than AMPH (*S*(+)-**3**). Likewise, *S*(-)-cathinone was more potent than ( $\pm$ )-cathinone and *R*(+)-cathinone both as a locomotor stimulant and in drug discrimination studies with AMPH-

**Fig. 2** Structures of ( $\pm$ )-cathinone (**1**), *S*(-)-cathinone or *S*(-)-**1**, *R*(+)-cathinone or *R*(+)-**1**, (+)-cathine (**2**), *S*(+)-amphetamine or *S*(+)-**3**, and *R*(-)-amphetamine or *R*(-)-**3**



trained and cathinone-trained rats. Hence, two of the earliest relevant SAR findings were that oxidation of the hydroxyl group of (+)cathine (**2**) to its corresponding keto analog (i.e., cathinone, **1**) resulted in retention of stimulant action and an increase in behavioral potency and that *S*(-)-cathinone was more potent as a stimulant or amphetamine-like agent than *R*(+)-cathinone.

The reduced *in vivo* potency of (+)cathine (**2**) relative to cathinone (**1**) might be attributed to its lower lipophilicity ( $c\text{Log}P = 0.81$  and  $1.16$ , respectively) and the consequent decreased ability of **2** to penetrate the blood-brain barrier, and/or because the hydroxyl group of **2** is simply not pharmacologically tolerated by its target protein.

Although the (+)-isomer of amphetamine is the more potent of its two optical isomers (i.e., it represents the *eutomer*), it is the (-)-isomer that represents the cathinone *eutomer*. Regardless, it is the absolute configuration of these isomers, the *S*-isomer in both cases (Fig. 2), not their optical rotation as designated by + or -, that most accurately describes their structural relationship in three-dimensional space.

Some of the next SAR questions addressed were (1) does the  $\alpha$ -methyl group of cathinone contribute to its stimulant/stimulus actions, (2) what is the effect of aryl substitution, and (3) will *N*-alkylation alter the potency of cathinone? Initially, because cathinone was a central stimulant, these questions were addressed by examining their locomotor actions in rodents and discriminative stimulus effects in rats (such studies are still being employed). As time went on, it was demonstrated that cathinone, like amphetamine, was a dopamine (DA) releasing agent, and later studies turned in that direction to investigate SAR.

*$\alpha$ -Demethylation:* As a locomotor stimulant in mice,  $\alpha$ -desmethylecathinone (**4**, Fig. 3), where the  $\alpha$ -methyl group of cathinone has been eliminated, was inactive at

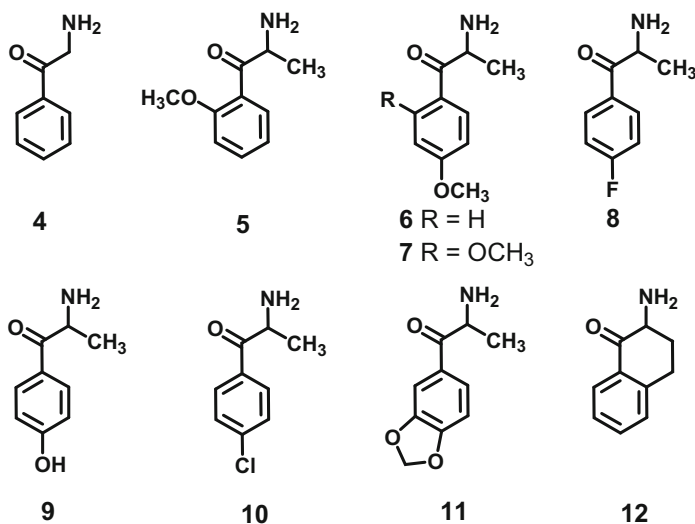


Fig. 3 Structures of some cathinone analogs involved in early SAR investigations

several times the effective dose of cathinone [5]. In drug discrimination studies, **4** failed to substitute (i.e., produced vehicle-appropriate responding) at doses of up to 10 times the ED<sub>50</sub> dose of *S*(–)cathinone (*S*(–)**1**) both in rats trained to discriminate AMPH [6] or (±)cathinone from saline vehicle [7, 8]. As a releasing agent of tritiated dopamine ([<sup>3</sup>H]DA) from rat caudate nucleus, α-desmethylecathinone (**4**) was about one-fourth as potent as *S*(–)cathinone [9]. Much more recently, it was shown that **4** is about one-third as potent as cathinone (EC<sub>50</sub> = 208 nM and 83 nM, respectively) as a DA releasing agent in a rat brain synaptosome preparation [10]. If the behavioral effects of cathinone are related to the release of dopamine via the dopamine transporter (DAT), as are those of amphetamine, some effect might have been expected for the doses examined; it would appear, then, that α-desmethylecathinone (**4**) either does not readily enter the brain and/or it is rapidly metabolized in vivo. It might be noted that β-phenylethylamine (PEA), the α-desmethyl analog of amphetamine, also failed to substitute in AMPH-trained rats [7] even though it is only about one-third as potent as AMPH as a depolarizing agent (i.e., the signature of a releasing agent) at the human dopamine transporter [11]. Reith et al. [10] demonstrated that PEA is about one-fourth as potent as AMPH as a dopamine releasing agent. Chain extension of **4** also resulted only in weakly active compounds [9].

*Aryl-substitution:* Relatively few ring-substituted cathinone analogs have been examined. 2-Methoxycathinone, 4-methoxycathinone, 2,4-dimethoxycathinone, and 4-fluorocathinone (**5–8**, respectively) failed to produce locomotor stimulation in mice at several times as an active dose of *S*(–)cathinone [5]. In rats trained to discriminate (±)cathinone from vehicle, 4-methoxycathinone (**6**) and 4-hydroxycathinone (**9**) produced saline-like effects, and 4-chlorocathinone (**10**) produced only partial generalization [7].

In AMPH-trained rats, 3,4-methylenedioxycathinone (MDC, **11**) elicited partial (50%) substitution, followed by disruption of the animals' behavior at slightly higher doses [12]. This was an indication that MDC (**11**) likely produces central effects other than, or in addition to, its AMPH-like action. Indeed, MDC (**11**) fully substituted in rats trained to discriminate the empathogen MDMA (i.e., 1-(3,4-methylenedioxyphenyl)-2-aminopropane, Ecstasy) from vehicle [12]. MDC (**11**) is the β-keto or β-carbonyl counterpart of MDA, and drug discrimination studies hinted that MDC might possess MDMA-like behavioral qualities.

*Conformational constraint:* 2-Amino-1-tetralone (**12**, Fig. 3) is an example of a conformationally constrained analog of cathinone. In rats trained to discriminate AMPH from saline vehicle, **12** produced saline-like responding at 10 times the ED<sub>50</sub> dose of *S*(–)cathinone; however, as a releasing agent of [<sup>3</sup>H]DA from rat caudate nucleus, it was only about five times less potent than *S*(–)cathinone [9].

*N-Alkylation:* Similar to amphetamine, cathinone is a central stimulant and a DA releasing agent. Because *N*-monomethylation of amphetamine, to afford methamphetamine, enhances its stimulant potency, *N*-(mono)methylcathinone was prepared and termed “methcathinone” (MCAT, **13**, Fig. 4) analogous to amphetamine/methamphetamine terminology [13]. Although this entity was first synthesized in the early twentieth century, and its locomotor stimulant actions in

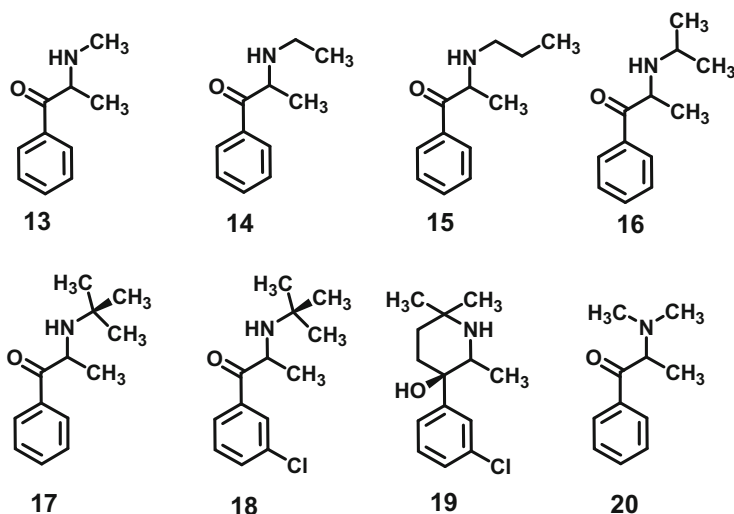


Fig. 4 Some amine-modified methcathinone (**13**) analogs

rodents were noted (reviewed: [4]), it was not until the term “methcathinone” was coined that **13** became a serious target of investigation. Shortly thereafter, it was found that methcathinone (**13**) constituted a serious drug abuse problem in the former Soviet Union where it was known as ephedrone; however, reports of its use had yet to be disseminated in the scientific literature (reviewed: [4]).

Methcathinone (**13**) was identified as a potent DA releasing agent, a locomotor stimulant in rodents, and as an agent more potent than methamphetamine in tests of stimulus generalization in drug discrimination studies (see Table 1) using AMPH-trained rats [12]. *S*(–)Methcathinone also substituted in *S*(+)methamphetamine and (–)ephedrine-trained rats [14, 15]. Behaviorally, *S*(–)methcathinone was found more potent than its *R*(+)-enantiomer in drug discrimination (Table 1) and mouse locomotor studies when enantiomeric comparisons were made.

Homologation of the *N*-methyl group of methcathinone (**13**) to an ethyl (i.e., ethcathinone) or *n*-propyl group (**14** and **15**, respectively, Fig. 4) resulted in small declines in potency in drug discrimination studies with rats trained to discriminate AMPH from vehicle (Table 1).

More recently, ethcathinone (**14**) was shown to behave both as a weak DA reuptake inhibitor ( $IC_{50} = 1014$  nM) and a weak DA releasing agent ( $EC_{50} = 2118$  nM), whereas its propyl homolog **15** was found inactive as a DA reuptake inhibitor [10].

*N*-Isopropylcathinone (**16**) and *N*-*tert*-butylcathinone (**17**) (Fig. 4) produced stimulant-characteristic hyperlocomotion in rats at doses of 7.5 mg/kg and 10 mg/kg, respectively, that were approximately half that seen following administration of methcathinone at 5 mg/kg [16]. Compound **17** is the des-chloro analog of the antidepressant bupropion (**18**, Fig. 4), and bupropion was also found to be a locomotor stimulant in the same study mentioned above at doses of 10 and

**Table 1** Comparison of stimulus generalization potencies of several amphetamine and cathinone analogs to substitute for training drug using rats trained to discriminate *S*(+) amphetamine from saline vehicle [12]

| Agent                                                  | ED <sub>50</sub> (mg/kg) |
|--------------------------------------------------------|--------------------------|
| (±)Amphetamine ( <b>3</b> )                            | 0.71                     |
| (±)Methamphetamine                                     | 0.49                     |
| (±)Methcathinone ( <b>13</b> )                         | 0.37                     |
| <i>S</i> (-) <b>13</b>                                 | 0.25                     |
| <i>R</i> (+) <b>13</b>                                 | 0.66                     |
| (±)Ethcathinone ( <b>14</b> )                          | 0.77                     |
| (±) <i>N</i> - <i>n</i> -Propylcathinone ( <b>15</b> ) | 2.02                     |
| (±) <i>N,N</i> -Dimethylcathinone ( <b>20</b> )        | 0.61                     |
| <i>S</i> (-) <b>20</b>                                 | 0.44                     |

15 mg/kg. In rats trained to discriminate AMPH from saline vehicle, bupropion (**18**) (ED<sub>50</sub> = 5.4 mg/kg) fully generalized to (+)amphetamine, but was 18 times lower in potency; of six bupropion metabolites, only one substituted for the AMPH stimulus: *S,S*-hydroxybupropion (**19**, Fig. 4) (ED<sub>50</sub> = 4.4 mg/kg) [17].

A number of years later, Carroll et al. [18] conducted a very thorough SAR investigation of three dozen bupropion analogs by examining their binding at the DAT, norepinephrine transporter (NET), and the serotonin (5-HT) transporter (SERT), their actions as reuptake inhibitors at all three major transporters, their locomotor stimulant actions in mice, and their stimulus generalization properties in rats trained to discriminate cocaine from vehicle. The majority of the analogs possessed the *tert*-butyl amine substituent common to bupropion (**18**). The des-chloro analog of bupropion (i.e., **17**) displayed about one-sixth the affinity of bupropion (**18**) at DAT, was equipotent at NET and inactive at SERT, was about half as potent as **18** as a locomotor stimulant, and substituted in cocaine-trained rats. Another interesting observation was that extension of the  $\alpha$ -methyl side chain had a pronounced influence on affinity at DAT with the following rank order of potency: -methyl < -ethyl < -*n*-propyl > *n*-butyl > *n*-pentyl > *n*-hexyl, with the *n*-hexyl analog still binding with an affinity comparable to bupropion (**18**). A conformationally constrained bupropion analog (i.e., the *N-tert*-butyl 5-chloro counterpart of **12**) displayed little affinity for DAT (or NET or SERT) but was twice as potent as **18** as a locomotor stimulant.

Together, these findings suggested that fairly bulky substituents are accommodated on the terminal amine of cathinone, but that they tended to decrease the potency of the resultant agents as amphetamine-like stimulants. At the time, little was known about the mechanism(s) of action of these agents.

*N,N*-Dimethylation of cathinone (i.e., *N,N*-dimethylcathinone or *N*-methylmethcathinone, **20**, Fig. 4) resulted in retention of AMPH-like stimulus action, but with about half the potency of methcathinone (**13**); the *S*(-)-isomer of **20** was more potent than racemic **20** (Table 1). It might be mentioned that **20**, also termed dimethylpropion, metamfepramone, and DMCN, was once examined as an anorectic agent; investigation of its metabolism in human subjects revealed that nearly half of an orally administered dose of **20** was metabolically demethylated to what is now termed methcathinone (**13**) [19]. Hence, some of the behavioral actions ascribed to **20** might be the result of its metabolism to **13**.

*Summary:* Learned from early SAR studies was that (1) oxidation of the  $\beta$ -hydroxyl group of (+)cathine to cathinone enhances its stimulant character; (2) cathinone is an amphetamine-like central stimulant with potency nearly comparable to, or greater than, that of amphetamine; (3) the *S*(–)-isomer of cathinone is more potent than its *R*(+)enantiomer in various behavioral assays; (4)  $\alpha$ -demethylation abolishes cathinone-like behavioral actions up to the doses evaluated, but results in an agent that retains DA releasing action; (5) aryl substitution, at least for those analogs examined, reduces or abolishes amphetamine-like stimulant/stimulus actions; (6) conformational constraint of the side chain of cathinone, as in 2-amino-1-tetralone (**12**), diminishes stimulant character; (7) *N*-monomethylation (viz. methcathinone, **13**) enhances the potency of cathinone in behavioral assays; (8) *S*(–)methcathinone is more potent than *R*(+)methcathinone in behavioral assays; (9) homologation and/or increasing the bulk of the *N*-methyl group of methcathinone to an ethyl, *n*-propyl, isopropyl, or *tert*-butyl group results in retention of stimulant character but in a reduction in potency; and (10) *N,N*-dimethylation of cathinone results in retention of amphetamine-like character, but with a slight decrease in potency. Early studies also provided evidence that cathinone (**1**) and methcathinone (**13**) behaved, at least in part, as DA releasing agents.

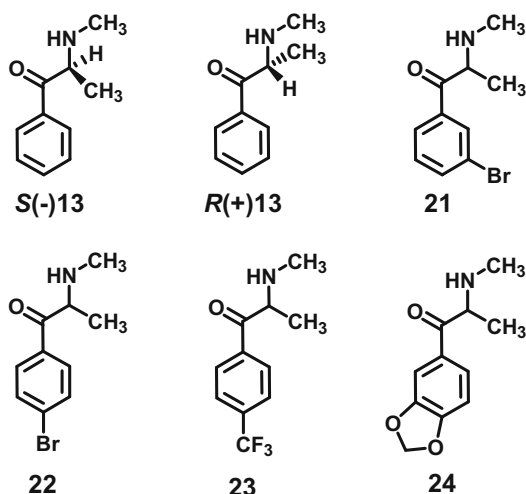
### 3 The Methcathinone Years

Until the mid-1990s, studies focused primarily on structural modification of cathinone; however, once methcathinone was identified as a potent central stimulant, there was a shift in attention to analogs of the latter. There was also a better understanding that methcathinone and some of its analogs might be acting as DA releasing agents. SAR derived from behavioral studies began to decline somewhat in favor of studies that focused more on the SAR for releasing action at the DAT. Eventually, methcathinone became a US Schedule I substance.

Due to the substantial potency of methcathinone (**13**) as a central stimulant, investigations were conducted on the stimulus generalization of methcathinone and its optical isomers *S*(–)**13** and *R*(+)**13** (Fig. 5) in cocaine-trained rats, the use of methcathinone as a potential training drug in drug discrimination studies, and on rodent locomotor studies on a few aryl-substituted methcathinone analogs.

In a locomotor assay in rats, 3-bromomethcathinone (**21**, Fig. 5) produced hyperlocomotion at doses of 7.5 and 10 mg/kg comparable to that produced by methcathinone at 5 mg/kg, whereas 4-bromomethcathinone (**22**), now termed brephedrone or 4-BMC, was inactive at these doses [16]. Subsequently, in a related assay in rats, 4-trifluoromethylmethcathinone (4-CF<sub>3</sub> MCAT, **23**) was inactive as a locomotor stimulant [20].

In cocaine-trained rats, stimulus generalization occurred with the following order of potency (ED<sub>50</sub> values given in parenthesis): *S*(–)MCAT (0.18 mg/kg) > ( $\pm$ )MCAT (0.39 mg/kg) > *R*(+)MCAT (0.51 mg/kg) > cocaine (2.6 mg/

**Fig. 5** Some early methcathinone analogs**Table 2** Biogenic amine releasing potency of several agents of interest [24]

| Agent                             | EC <sub>50</sub> (nM) |       |          |
|-----------------------------------|-----------------------|-------|----------|
|                                   | DAT                   | NET   | SERT     |
| S(+)-Amphetamine <b>S(+)-3</b>    | 24.8                  | 7.1   | 1765     |
| S(+)-Methamphetamine              | 24.5                  | 12.3  | 736      |
| S(-)-Cathinone <b>S(-)-1</b>      | 18.5                  | 12.4  | 2386     |
| S(-)-Methcathinone <b>S(-)-13</b> | 14.8                  | 13.1  | 1772     |
| (+)-Cathine <b>2</b>              | 88.3                  | 15.0  | Inactive |
| (-)-Cathine <b>25</b>             | 1371.0                | 137.0 | Inactive |

kg) [21]. Later, Kohut et al. [22] showed that methcathinone also substituted for cocaine in monkeys.

Unlike MDC (**11**), methylenedioxymethcathinone (MDMC, methylone, **24**), the *N*-methyl analog of MDC, substituted in AMPH-trained rats; however, it was six times less potent than methcathinone [12]. MDMC (**24**) also substituted in MDMA-trained rats [12].

S(-)-Methcathinone was demonstrated to serve as a training drug in rats, and stimulus generalization occurred upon administration of other central stimulants with the following order of potency (ED<sub>50</sub> values given in parentheses): S(-)MCAT (0.11 mg/kg; 0.5 μM/kg) > S(+)-methamphetamine (0.17 mg/kg, 0.9 μM/kg) > (±)MCAT (0.25 mg/kg, 1.2 μM/kg) > R(+)-MCAT (0.43 mg/kg, 2.1 μM/kg) ~ (±)cathinone (0.41 mg/kg, 2.2 μM/kg) [23]. As an aside, it might be noted that the S(-)-methcathinone stimulus also generalized to cocaine (ED<sub>50</sub> = 1.47 mg/kg, 4.3 μM/kg) [23].

Because methcathinone (**13**) had been shown to act as a releasing agent at the DAT, a SAR study was conducted, and several agents were compared for their ability to release DA, norepinephrine, and 5-HT from rat brain synaptosomes [24]. Some of the data are shown in Table 2.

The behaviorally more potent *S*-isomers of cathinone (**1**), methcathinone (**13**), amphetamine (**3**), and methamphetamine displayed comparable potencies, and similar potencies as releasing agents, at DAT and NET (Table 2). All displayed lower potencies as 5-HT releasing agents. The potency of (+)cathine (**2**) as a norepinephrine releasing agent was comparable to that of the above agents; however, **2** was severalfold less potent than the others at DAT and inactive at SERT (Table 2). Data for (–)cathine (**25**) is shown in Table 2 for comparison. Interesting is that an AMPH stimulus generalized to all of the agents in Table 2 except for (–)cathine (**25**) [25] supporting a possible role for DA and/or norepinephrine in their actions. A decade later, Cozzi et al. [20] compared the actions of 4-CF<sub>3</sub> MCAT (**23**) with those of its 2- and 3-substituted positional isomers; introduction of the –CF<sub>3</sub> group had a deleterious effect on DAT and NET release and resulted in enhanced selectivity for SERT, and **23** was found to lack activity as a locomotor stimulant in rats (the other two positional isomers were not examined).

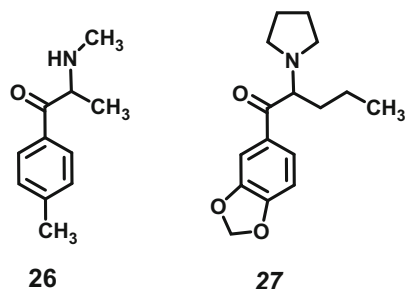
From these investigations, it was (1) confirmed that *S*(–)methcathinone is more potent than *R*(+)methcathinone as a central stimulant/stimulus, (2) shown that *S*(–)methcathinone is a potent DA and norepinephrine releasing agent, (3) demonstrated that introduction of aryl substituents decreases the stimulant and DAT releasing effects of methcathinone, and (4) shown that *S*(–)methcathinone can be used as a training drug to examine the stimulus effects of other cathinone and non-cathinone central stimulants.

## 4 Current SAR Studies

In 2010, Iversen [26] submitted a report to the British Home Office on the alarming emergence of synthetic cathinone analogs on the European clandestine market. This ushered in a new era in cathinone research. A recent PubMed search (accessed December 20, 2015) for “synthetic cathinones” yielded 189 papers published during the 5-year period between January 2011 and December 2015.

One early drug combination popular around 2010 was referred to as *bath salts*; it was also known by several other names. *Bath salts* contained either methylone (MDMC, **24**), mephedrone (**26**), methylenedioxypyrovalerone (MDPV, **27**) (Fig. 6), or a combination of one, two, or more of these and/or other agents

**Fig. 6** Two early “bath salts” constituents: mephedrone (**26**) and MDPV (**27**)



(reviewed: [4]). Some information was already available about methylone (**24**) (*vide supra*), but little was known about the other two agents. Based on SAR already formulated for cathinone and amphetamine analogs, it was suspected that mephedrone (**26**) would be a DA releasing agent with reduced potency and selectivity relative to methcathinone (**13**). MDPV (**27**) represented something “new,” although Meltzer et al. [27] had already examined several related pyrovalerones (but not MDPV) as DA reuptake inhibitors for their therapeutic potential.

Various investigators demonstrated that these three synthetic cathinones (methylone, mephedrone, MDPV), among others (see below), behaved as locomotor stimulants in rodents (e.g., [28–34]), were self-administered by rats [29, 35, 36], and produced discriminative stimulus effects similar to other central stimulants using methamphetamine-trained and cocaine-trained rats [32, 37] and MDPV-trained mice [31]. There is substantial difficulty in formulating a reliable SAR for these agents (not the intent of the original investigations and very difficult in retrospect) because the studies were not focused on SAR; this is related to the paucity and structural diversity of agents examined in the individual studies, the different animal species employed, the different routes (e.g., *i.v.* versus *i.p.* or *i.m.*) of drug administration, and the various temporal parameters employed. Gatch et al. [37] made the observation that although locomotor activity was a good predictor for dose ranges to be examined in their subsequent drug discrimination studies, the magnitude of locomotor stimulation might not be a good predictor of abuse liability for the agents they examined. They also speculated that reaching a threshold level of neurotransmitter release or reuptake might be sufficient to produce behavioral effects, but that subtle differences might not be important.

Attention turned to the ability of synthetic cathinones to act at biogenic amine neurotransmitter transporters (i.e., DAT, NET, SERT) that might underlie their behavioral effects. Mephedrone (**26**) was shown to behave as a DA releasing agent. Within a year after the Iversen [26] report, MDPV (**27**) was shown to display characteristics of a DA reuptake inhibitor. Using a frog oocyte preparation transfected with hDAT, mephedrone (**26**) produced dopamine-like depolarization, whereas MDPV (**27**) produced cocaine-like hyperpolarization [38–40]. These are the signatures of a releasing agent and a reuptake inhibitor, respectively. Simmler et al. [41] later examined several synthetic cathinones and found that mephedrone (**26**) was nearly equipotent as an inhibitor and releaser of DA and 5-HT, but substantially more potent as an inhibitor of NET. In contrast, MDPV (**27**) was a potent reuptake inhibitor at DAT and NET, a very weak inhibitor of SERT, but did not release DA or 5-HT [41]. Eshleman et al. [42] reported similar results. These overall findings were similar to what was reported by Baumann and co-workers [30] (Table 3), except that methylone (MDMC, **24**) was more potent as a DA releasing agent than a reuptake inhibitor. Different investigators employed different assays (procedures, radioligands, etc.) – hence, differences exist with respect to the results shown here. So again, there are problems to formulate reliable SAR between studies.

**Table 3** Effect of several synthetic cathinones on synaptosomal release and reuptake inhibition at biogenic transporters; data for amphetamine and cocaine included for comparison [30]

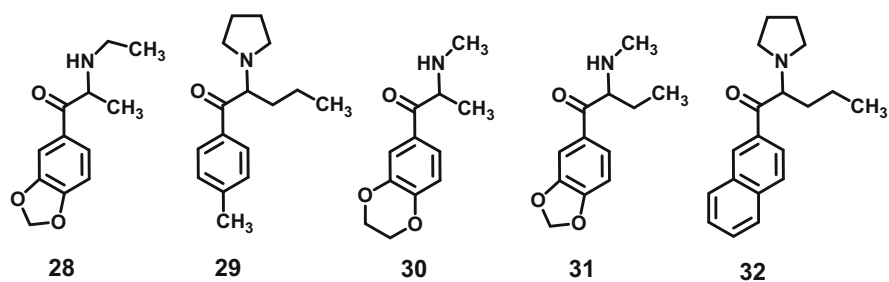
|                          | Release EC <sub>50</sub> (nM) |      |      | Reuptake inhibition IC <sub>50</sub> (nM) |      |      |
|--------------------------|-------------------------------|------|------|-------------------------------------------|------|------|
|                          | DAT                           | NET  | SERT | DAT                                       | NET  | SERT |
| Methylone ( <b>24</b> )  | 117                           | 140  | 234  | 1232                                      | 1031 | 1017 |
| Mephedrone ( <b>26</b> ) | 51                            | 58   | 122  | 762                                       | 487  | 422  |
| MDPV ( <b>27</b> )       | n.a.                          | n.a. | n.a. | 4.1                                       | 26   | 3349 |
| S(+)-Amphetamine         | 5.8                           | 6.6  | 698  | 93                                        | 67   | 3418 |
| Cocaine                  | n.a.                          | n.a. | n.a. | 211                                       | 292  | 313  |

*n.a.* not active, demonstrating release <35%

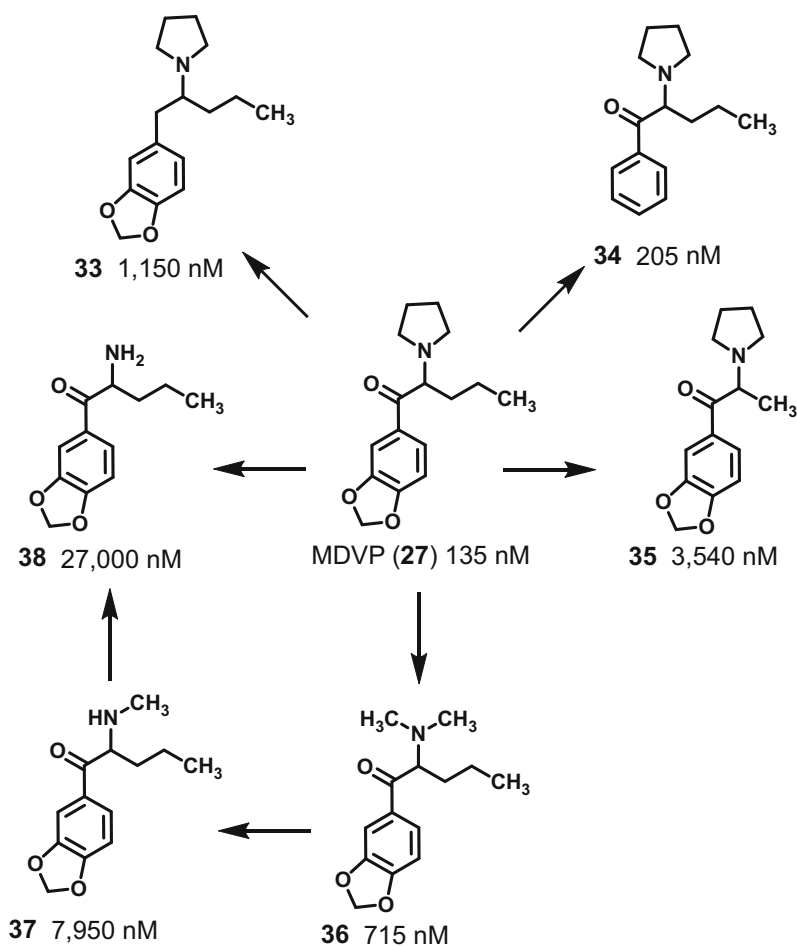
Expansion of the methylenedioxy ring of methylone (**24**) to an ethylenedioxy ring (i.e., ethylenedioxymethcathinone, EDMC, **30**, Fig. 7) decreased its potency as a releasing agent at all three transporters by two- to threefold [43]. Homologation of the  $\alpha$ -methyl group of methylone (**24**) to an  $\alpha$ -ethyl group (i.e., butylone, **31**) and replacement of the methylenedioxy group of MDPV (**27**) with a fused phenyl ring (i.e., naphyrone, **32**) resulted in reuptake inhibitors at the three transporters [41, 42]. Naphyrone (**32**) was five- to tenfold more potent than butylone (**31**) at DAT, NET, and SERT and five- to tenfold less potent than MDPV (**27**) at DAT and NET, but 10 times more potent than MDPV (**27**) at SERT. It was apparent from these (and other concurrent or subsequent) investigations that substituents on the terminal amine and/or at the  $\alpha$ -position of cathinone analogs have a significant impact both on the actions of these agents as releasing agents (i.e., as substrates) versus reuptake inhibitors, and on their selectivity for the major biogenic amine transporters.

*Synthetic cathinones as reuptake inhibitors:* MDPV (**27**) was unique among the synthetic cathinones appearing on the clandestine market because it was the first of them to be identified as a DA reuptake inhibitor. Figure 8 shows a systematic SAR deconstruction of MDPV (**27**) to determine which of, and to what extent, its various structural features contribute to its actions as a DA reuptake inhibitor [44]. All of the compounds shown in Fig. 8 behaved as reuptake inhibitors but varied appreciably with respect to potency. Removal of the carbonyl group, converting MDPV to its amphetamine analog **33**, reduced its potency by about eightfold, whereas removal of the methylenedioxy substituent (i.e.,  $\alpha$ -PVP, **34**) had a negligible effect. The length of the  $\alpha$ -side chain would appear to be critical; shortening the side chain (i.e., MDPPP, **35**) resulted in a > 25-fold decrease in potency. With an intact side chain, the next most important feature was the amine. Conversion of the amine from the simplest tertiary amine (i.e., **36**, dimethylone) to a secondary or primary amine (i.e., **37** or pentylone and **38**, respectively) ultimately resulted in a 200-fold decrease in potency).

Because the methylenedioxy group played a minimal role in the ability of MDPV (**27**) to act as a DAT inhibitor, a series of analogs lacking this functionality was examined with a focus on SAR [47]. These analogs might be viewed as being derived from  $\alpha$ -PVP (*flakka*, **34**) (Fig. 9 and Table 4) – currently, a very popular



**Fig. 7** Structures of ethylone (28), pyrovalerone (29), EDMC (30), butylone (31), and naphyrone (32)



**Fig. 8** Deconstruction of MDPV (27). Values represent the potency of the analogs to block the reuptake of DA [44]

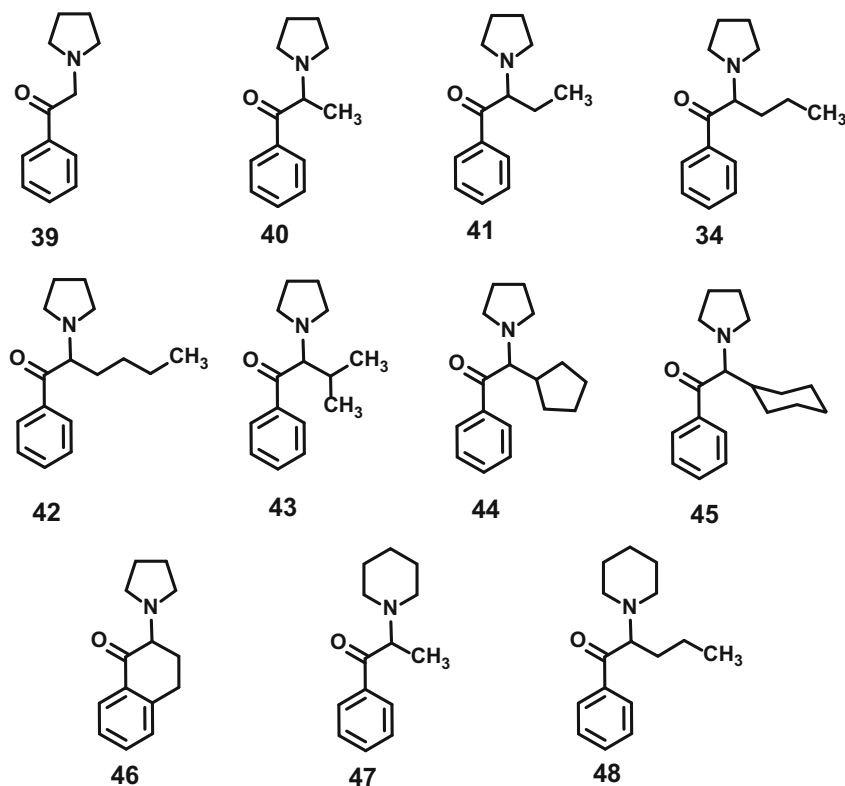
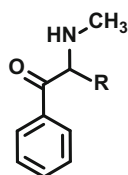


Fig. 9 Deconstructed (39–41) and elaborated (42–48) analogs of  $\alpha$ -PVP (34)

drug of abuse. Using the deconstruction process, the  $\alpha$ -*n*-propyl group of **34** was shortened in a stepwise manner [47]. Truncation of the  $\alpha$ -propyl substituent to an  $\alpha$ -ethyl group (i.e.,  $\alpha$ -PBP, **41**, Table 4) reduced potency by about threefold, and further contraction to an  $\alpha$ -methyl group (i.e.,  $\alpha$ -PPP, **40**) resulted in an overall >tenfold decrease in potency. Elimination of the  $\alpha$ -*n*-propyl group altogether – that is, replacement by  $-H$  (i.e., **39**, Table 4) – resulted in a nearly 200-fold decrease in potency. Nevertheless, all of the analogs behaved as DA reuptake inhibitors. The findings support those shown in Fig. 8 in that the  $\alpha$ -substituent of synthetic cathinones plays a major role in their actions as reuptake inhibitors at DAT when the amine substituent is held constant as a pyrrolidine moiety. In addition, none of the analogs was effective as a reuptake inhibitor at SERT ( $IC_{50} > 10,000$  nM). From these data, it can be surmised that the length of the  $\alpha$ -substituent is influential for DAT action, but that action at SERT might not readily accommodate a pyrrolidine moiety regardless of the length of the  $\alpha$ -substituent.

Because the length/bulk of the  $\alpha$ -substituent seemed important for these agents to act as reuptake inhibitors at DAT, additional compounds were examined (i.e., an

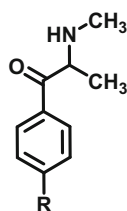
**Table 4** Potency of deconstructed and elaborated  $\alpha$ -PVP (**34**) analogs to inhibit synaptosomal reuptake at DAT [47]

| Agent     | R                                                                | IC <sub>50</sub> (nM) |
|-----------|------------------------------------------------------------------|-----------------------|
| <b>39</b> | –H                                                               | 3250                  |
| <b>40</b> | –CH <sub>3</sub>                                                 | 196.7                 |
| <b>41</b> | –CH <sub>2</sub> CH <sub>3</sub>                                 | 63.3                  |
| <b>34</b> | –CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>                 | 17.5                  |
| <b>42</b> | –CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub> | 11.6                  |
| <b>43</b> | –CH(CH <sub>3</sub> ) <sub>2</sub>                               | 92.3                  |
| <b>44</b> | –C <sub>5</sub> H <sub>9</sub>                                   | 17.1                  |
| <b>45</b> | –C <sub>6</sub> H <sub>11</sub>                                  | 8.3                   |

“elaboration” investigation – see Glennon and Young [1] for conceptual details). Increasing the length of the  $\alpha$ -substituent from *n*-propyl (i.e., **34**) to *n*-butyl (i.e.,  $\alpha$ -PHP, **42**) resulted in a slight increase in potency (Table 4). Branching of the  $\alpha$ -ethyl side chain of **41** to its isopropyl counterpart **43** reduced potency by about 50% (Table 4). But, as might now be expected based on the information provided in Table 4, increasing the bulk of this branched analog should result in increased potency. Elaborated analogs **44** and **45** (IC<sub>50</sub> = 17.1 and 8.3 nM, Table 4) were at least as potent as  $\alpha$ -PVP (**34**) [47].

Compound **46** is a conformationally constrained analog of **41**; its potency (IC<sub>50</sub> = 12,900 nM) is >200-fold less than that of **41**. Ring expansion of the pyrrolidine ring of  $\alpha$ -PVP (**34**) to a piperidine ring (i.e., **48** IC<sub>50</sub> = 128 nM) resulted in a sevenfold decrease in potency; likewise, the piperidine analog of **40** (i.e., **47** IC<sub>50</sub> = 2490 nM) also displayed reduced potency. The overall results of these studies are not inconsistent with results published by Meltzer et al. [27] on a related series of pyrovalerones although, in their studies, most of the compounds possessed aryl substituents such that direct SAR comparisons are difficult to make.

Pyrovalerone (**29**) possesses a chiral center and two optical isomers are possible; the *S*-isomer was 100 times more potent than its *R*-enantiomer as a reuptake inhibitor at DAT [27]. Both isomers were less potent at NET and SERT. MDPV (**27**) also exists as a pair of optical isomers (Fig. 10), and both were prepared and examined [45] with respect to their neurochemical actions on neurotransmitter reuptake and behavioral effects in an assay of intracranial self-stimulation (ICSS) in rats – a behavioral procedure used to evaluate abuse potential. In assays of DAT uptake inhibition, *S*(+)MDPV (IC<sub>50</sub> = 2.13 nM) was twice as potent as ( $\pm$ )MDPV (IC<sub>50</sub> = 4.85 nM) and 180-fold more potent than *R*(–)MDPV (IC<sub>50</sub> = 382.80 nM); as such, the *S*-isomer was 100-fold more potent than cocaine (IC<sub>50</sub> = 198.80 nM).

**Table 5** Potency of selected 4-substituted methcathinone analogs as releasing agents at DAT and SERT, and in a rat ICSS assay, used in a QSAR study [46]

| Agent                                        | R                 | DAT<br>EC <sub>50</sub><br>(nM) <sup>a</sup> | SERT<br>EC <sub>50</sub><br>(nM) <sup>a</sup> | DAT<br>selectivity <sup>b</sup> | ICSS<br>maximum %<br>baseline<br>facilitation <sup>c</sup> |
|----------------------------------------------|-------------------|----------------------------------------------|-----------------------------------------------|---------------------------------|------------------------------------------------------------|
| Methcathinone ( <b>13</b> )                  | –H                | 12.5                                         | 3860                                          | 309                             | 191.9                                                      |
| Flephedrone ( <b>50</b> )                    | –F                | 83.4                                         | 290                                           | 15.4                            | 156.3                                                      |
| Methedrone ( <b>51</b> )                     | –OCH <sub>3</sub> | 506                                          | 120                                           | 0.24                            | 110.9                                                      |
| 4-Chloromethcathinone ( <b>52</b> )          | –Cl               | 42.2                                         | 144                                           | 3.40                            | 114.9                                                      |
| 4-Bromomethcathinone ( <b>22</b> )           | –Br               | 59.4                                         | 60                                            | 1.01                            | 118.0                                                      |
| Mephedrone ( <b>26</b> )                     | –CH <sub>3</sub>  | 49.1                                         | 118                                           | 2.41                            | 102.5                                                      |
| 4-Trifluoromethylmethcathinone ( <b>23</b> ) | –CF <sub>3</sub>  | 2700                                         | 190                                           | 0.07                            | 90.9                                                       |

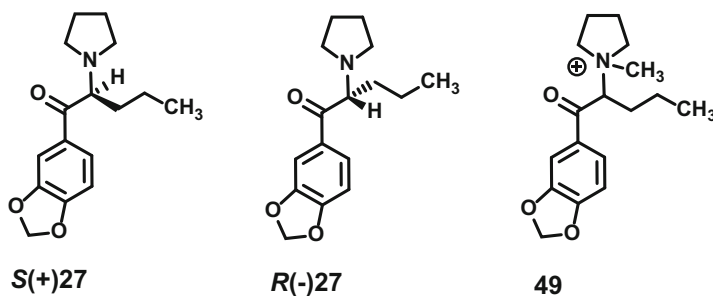
<sup>a</sup>EC<sub>50</sub> values and ICSS data are from Bonano et al. [49]

<sup>b</sup>DAT selectivity calculated as (DAT EC<sub>50</sub>)<sup>–1</sup> ÷ (SERT EC<sub>50</sub>)<sup>–1</sup>; higher values indicate greater DAT selectivity

The three were less potent at NET uptake inhibition, but with the same rank order of potency, IC<sub>50</sub> = 9.86 nM, 16.84 nM, and 726 nM, respectively, relative to cocaine IC<sub>50</sub> = 395.9 nM. Neither (±)MDPV nor either of its optical isomers inhibited the reuptake at SERT. *S*(+)-MDPV produced an abuse-related and dose-dependent facilitation of ICSS in rats, and the potency of *S*(+)-MDPV (significant facilitation at doses ≥0.1 mg/kg) was greater than that of (±)MDPV, whereas *R*(–)-MDPV failed to alter ICSS at doses up to 100 times greater than the lowest effective dose of *S*(+)-MDPV [45].

Another preliminary finding, although additional studies are required, was that the *N*-methyl quaternary amine counterpart of MDPV (i.e., Q-MDPV, **49**, Fig. 10) produced hyperpolarization in frog oocytes transfected with hDAT ([48], Sakloth, Solis, DeFelice, Glennon, *unpublished data*). These results suggest that **49** can act as a DAT reuptake inhibitor.

One of the first QSAR studies published on synthetic cathinones to inhibit reuptake at DAT indicated that potency is related to the “size” of the α-side chain [46, 48]. That is, using the data shown for the eight agents in Table 4, potency was significantly correlated both with the volume ( $r = 0.909$ ) and the lipophilicity ( $\pi$  value,  $r = 0.917$ ) of their α-substituents. However, for the substituents in this set,



**Fig. 10** Newer MDPV (**27**) analogs: *S*(+)-MDPV, *R*(-)-MDPV, and Q-MDPV (**49**)

volume and  $\pi$  were highly intercorrelated ( $r = 0.997$ ). Additional studies will be required to determine which of these two parameters is more important for activity and, obviously, to determine the optimal volume and/or lipophilicity for this action.

*Synthetic cathinones as releasing agents:* As already mentioned, mephedrone (**26**), although not as potent or selective as MCAT (**13**), was found to act as a releasing agent at DAT. The same is true of a number of related methcathinone analogs. The literature has described a number of studies on such agents (e.g., see [4] for a review and more recent references on individual synthetic cathinones). SAR and QSAR endpoints were not the focus of most of these investigations, and only those that specifically addressed the topic will be mentioned here.

Seven methcathinone analogs that differed only with respect to their 4-position substituents were examined for their ability to modulate *in vivo* ICSS in rats and to act as substrates (i.e., releasers) at DAT and SERT. The potencies and selectivities of these agents varied over a broad range (Table 5). The most potent analog in the ICSS assay was MCAT (**13**), whereas the least potent was 4-trifluoromethylmethcathinone (**23**); the potencies of the other agents fell somewhere in between. *In vitro* DAT versus SERT selectivity correlated with *in vivo* efficacy to produce ICSS facilitation ( $r = 0.92$ ). Furthermore, the Taft steric parameter (i.e.,  $E_s$  value) of the 4-position substituents correlated both with DAT versus SERT selectivity ( $r = 0.78$ ) and magnitude of ICSS facilitation ( $r = 0.81$ ) [49]. There was no relationship either in ICSS facilitation, DAT potency, or SERT potency and either the electronic ( $\sigma$ ) or lipophilic ( $\pi$ ) character of the substituents. In a follow-up study, more specific steric parameters were examined including substituent volume and Verloop size (i.e., substituent length,  $L$ , and minimum or maximum substituent width,  $B1$  and  $B5$ , respectively). Maximal ICSS facilitation was negatively correlated with the four steric parameters: volume ( $\text{\AA}^3$ ) ( $r = -0.915$ ), length ( $L$ ) ( $r = -0.773$ ), minimum width ( $B1$ ) ( $r = -0.778$ ), and maximum width ( $B5$ ) ( $r = -0.814$ ). Internal correlations were found between certain parameters: volume and both substituent length ( $r = 0.814$ ) and maximal width ( $r = 0.935$ ), as well as between length and maximal width ( $r = 0.798$ ) [46]. The potency of the agents to promote *in vitro* monoamine release via DAT was negatively correlated with increasing volume ( $r = -0.803$ ) and maximal

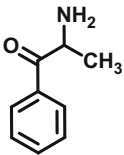
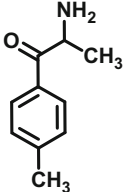
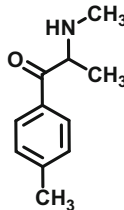
substituent width ( $r = -0.807$ ), whereas potency at SERT was positively correlated with increasing volume ( $r = 0.825$ ) and length ( $r = 0.903$ ) of the 4-position substituent. Selectivity for DAT vs. SERT was correlated with volume ( $r = -0.972$ ) and maximal width ( $r = -0.917$ ).

It might be expected that multiple structural features such as the terminal amine and the aromatic ring contribute to the interactions of MCAT analogs at DAT and SERT; but these structural features are common to all the analogs shown in Table 5. That is, these analogs varied only by the substituent at the 4-position; in this respect, they represent a matched set with only a single variable substituent among them. Found was that as the size of the 4-position substituent increased, potency at DAT decreased, whereas potency at SERT increased, and this reciprocal relationship was also seen with DAT versus SERT selectivity. It would seem, then, that the steric properties of the 4-position substituent play a major role in the actions of the investigated MCAT analogs. However, because of internal correlations between some of the steric parameters, it was not possible to identify a single steric parameter as being the most relevant. Nevertheless, it was speculated that because of the consistent identification and the higher correlation coefficients associated with steric volume (i.e., Å<sup>3</sup>), the total volume of the 4-position substituent is likely the most important feature for these interactions [46].

Homology modeling studies provided new models for hDAT and hSERT using the dDAT crystal structure as a template. Docking studies with the agents in Table 5 showed that large substituents at the MCAT (**13**) 4-position were better accommodated by hSERT than hDAT [46]. The results were consistent with the results of the QSAR studies described above. Furthermore, a hydrophobic interaction (HINT) analysis that considered potential interactions of the 4-position substituents with specific nearby transporter amino acid residues suggested that hydrophobic interactions provided by the 4-position substituent are necessary for potency at hSERT, whereas unfavorable polar interactions at the 4-position might play a role in determining potency at hDAT. The overall conclusion was that in the MCAT binding pocket associated with the 4-position substituent in hDAT, bulky substituents are not readily accommodated, whereas the larger and less polar binding pocket in hSERT more readily accommodated them. The overall conclusions of these QSAR and modeling/docking investigations are that (1) MCAT analogs with small 4-position substituents favor binding at DAT versus SERT, (2) larger substituents favor binding at SERT, and (3) the hydrophobic nature of these substituents modulates potency at SERT.

In a follow-up study, the 4-tert-butyl analog of methcathinone was prepared and examined. Although the potency of this agent as a DA releasing agent ( $EC_{50} = 942$  nM) was greater than that of its corresponding 4-trifluoromethyl counterpart **23**, it was only a partial (ca 50%) releasing agent; furthermore, this agent now acted as a weak reuptake inhibitor at DAT ( $IC_{50} = 2207$  nM) and lacked action as either a releasing agent or reuptake inhibitor at SERT up to concentrations of 1000 and 10,000 nM ([48], Sakloth, Partilla, Bauman, Glennon, *unpublished data*). The results support the finding that large 4-position substituents are not tolerated at DAT and that there is a limit to the size of this substituent that can be accommodated by SERT.

**Table 6** Potencies of stereoisomers of several simple cathinone analogs for the synaptosomal release of neurotransmitter from DAT, NET, and SERT [50, 51]

|                                                                                   |                          |                                                                                   |         |                                                                                   |                          |
|-----------------------------------------------------------------------------------|--------------------------|-----------------------------------------------------------------------------------|---------|-----------------------------------------------------------------------------------|--------------------------|
|  |                          |  |         |  |                          |
| <b>Cathinone</b>                                                                  | <b>4-Methylcathinone</b> | <b>Mephedrone</b>                                                                 |         |                                                                                   |                          |
|                                                                                   |                          | EC <sub>50</sub> (nM) <sup>a</sup>                                                |         |                                                                                   | DAT vs. SERT selectivity |
|                                                                                   | DAT                      | NET                                                                               | SERT    |                                                                                   |                          |
| <i>S</i> (–)Cathinone <i>S</i> (–) <b>1</b>                                       | 25                       | 14                                                                                | 9267    | 370                                                                               |                          |
| <i>R</i> (+)Cathinone <i>R</i> (+) <b>1</b>                                       | 184                      | 72                                                                                | >10,000 | >50                                                                               |                          |
| <i>S</i> (–)4-Methylcathinone <i>S</i> (–) <b>53</b>                              | 150                      | 89                                                                                | 179     | 1.2                                                                               |                          |
| <i>R</i> (+)4-Methylcathinone <i>R</i> (+) <b>53</b>                              | 391                      | 115                                                                               | 1592    | 4.1                                                                               |                          |
| <i>S</i> (–)Mephedrone <i>S</i> (–) <b>26</b>                                     | 74                       | –                                                                                 | 61      | 0.8                                                                               |                          |
| <i>R</i> (+)Mephedrone <i>R</i> (+) <b>26</b>                                     | 31                       | –                                                                                 | 1470    | 47                                                                                |                          |

<sup>a</sup>Some values have been rounded off to the nearest whole number

In a QSAR study, using *in vivo* microdialysis to examine the relationship between the volume of the 4-position substituent and the *in vivo* neurochemical selectivity of cathinone analogs to alter nucleus accumbens (NAc) DA and 5-HT levels, rats were implanted with bilateral guide cannulae targeting the NAc and were administered MCAT (**13**) and five of the 4-substituted MCAT analogs shown in Table 5 (i.e., **22**, **26**, **50–52**). All six compounds produced dose-dependent increases in NAc DA and/or 5-HT levels. *In vivo* selectivity (determined as the dose required to increase peak 5-HT levels by 250% divided by the dose required to increase peak DA levels by 250%) was correlated with *in vitro* selectivity to promote monoamine release via DAT and SERT ( $r = 0.95$ ) and the molecular volume (i.e., Å<sup>3</sup>) of the 4-position substituent ( $r = -0.85$ ). The results further support a relationship between these molecular, neurochemical, and behavioral measures described above [52].

Racemates, by definition, consist of an *equal* mixture of two optical isomers or antipodes of a given agent. Often, one isomer is the “active” isomer (i.e., the eutomer), whereas the other is less active or “inactive” (i.e., the distomer). When the distomer is inactive, the action is termed *stereospecific* (or, *enantiospecific*), and the eutomer is twice as potent as the racemate. The “inactive” isomer simply dilutes the potency of the “active” isomer by 50%, and the maximal theoretical potency of the eutomer is twice that of the racemate. In other cases, both isomers – the eutomer and the distomer – are “active,” but one is more potent than the other; the optical isomers are then said to produce a *stereoselective* (or *enantioselective*) effect.

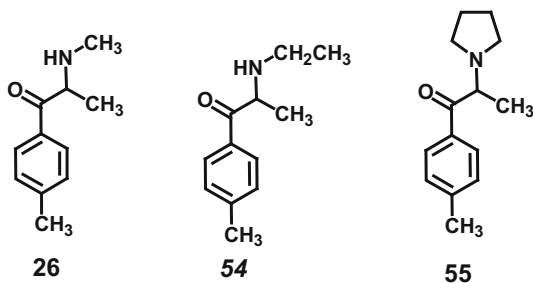
As described above, methcathinone (**13**) is stereoselective with respect to its behavioral actions and potency as a DA releasing agent; *S*(–)methcathinone

represents the eutomer. Mephedrone (**26**) is a less-selective releasing agent (Table 5). Mephedrone (**26**), which is 4-methylmethcathinone, can be deconstructed to 4-methylcathinone (**53**) and, by removal of the *N*-methyl group, to cathinone (**1**). An examination of the optical isomers of these agents can provide insight as to the stereoselective versus stereospecific nature of their actions. *S*(–) Cathinone (Table 6) is stereoselective, but not selective with respect to release at DAT versus NET. That is, both isomers are “active,” but the *S*(–)-isomer is more potent than its *R*(+) enantiomer by severalfold (Table 6). However, both cathinone isomers are essentially inactive at SERT. Introduction of the 4-methyl group (i.e., 4-methylcathinone, **53**) resulted both in decreased (<threefold) stereoselectivity for DAT/NET release and decreased selectivity for DAT/NET release versus release at SERT (Table 6). Introduction of an *N*-methyl group (i.e., mephedrone, **26**) resulted in further decreased stereoselectivity at DAT with the two optical isomers being nearly equipotent (i.e., the difference in potency for *S*(–)- and *R*(+) mephedrone is not much more than twofold). In addition, *S*(–) mephedrone is no longer selective for DAT versus SERT.

The abuse-related potential of biogenic amine releasing agents appears to be related to their ability to promote greater release via DAT than via SERT such that DAT-selective agents possess higher abuse liability (see [51, 53] for extended discussion). Consistent with this concept is that *R*(+) mephedrone with 50-fold selectivity for DAT over SERT produced in rats greater locomotor stimulation and rewarding properties as measured by conditioned place preference and facilitation of ICSS than its *S*(–) enantiomer which produced weak locomotor stimulation and lacked rewarding properties [50]. For 4-methylcathinone (**53**), *R*(+) **53** was less potent than its *S*-enantiomer to promote release at DAT, but displayed slightly greater DAT versus SERT selectivity and produced abuse-related effects in ICSS [51]. These studies (i.e., those reported by [50, 51]) were the first to show the subtle stereochemical relationship between the ability of optical isomers of cathinone analogs to behave as substrates at DAT and SERT and their stimulant or rewarding actions. It would seem that future studies should focus greater attention on the optical isomers of related agents.

As already alluded to, the nature of the terminal amine,  $\alpha$ -substituents, aryl substituents, and stereochemistry can alter the actions of synthetic cathinones. That is, methcathinone (**13**) is primarily a releasing agent at DAT, whereas introduction of a 4-methyl group (i.e., mephedrone, **26**) enhances its potency as a releasing agent at SERT such that methcathinone displays >300-fold selectivity for DAT, whereas mephedrone displays only slightly more than twofold selectivity (Table 5). Increasing the bulk on the terminal amine of cathinone analogs tends to enhance action as a DAT reuptake inhibitor (see Fig. 8). Also, the *N*-ethyl homolog of methcathinone, ethcathinone (**14**), is both a weak DA releasing agent and reuptake inhibitor [10]. Hence, by “mixing and matching” of appropriate substituents, certain cathinone analogs might possess “mixed” or “hybrid” actions. Saha et al. [54] examined this by evaluating three agents with gradually increasing steric bulk on the terminal amine: mephedrone (**26**), its *N*-ethyl homolog 4-methyl-*N*-ethylcathinone (**54**, 4-MEC), and its pyrrolidine counterpart 4'-methyl- $\alpha$ -pyrrolidinopropiophenone (**55**, 4-MePPP) (Fig. 11).

**Fig. 11** A structural comparison of mephedrone (**26**), 4-MEC (**54**), and 4-MePPP (**55**)



Mephedrone (**26**) and 4-MEC (**54**) were nearly equipotent at inhibiting reuptake at DAT ( $IC_{50}$  ca. 800 nM) and SERT ( $IC_{50}$  ca. 500 nM), whereas 4-MePPP (**55**) was more potent as an inhibitor at the former ( $IC_{50}$  = 215 nM) as opposed to the latter ( $IC_{50}$  > 10,000 nM). However, in a synaptosomal release assay, mephedrone (**26**) and 4-MEC (**54**) were similar in potency to evoke release from SERT ( $EC_{50}$  ca. 100 nM), whereas 4-MePPP (**55**) was inactive. In contrast, mephedrone was an effective releaser at DAT ( $EC_{50}$  = 39 nM), whereas 4-MEC (**54**) and 4-MePPP (**55**) were inactive. The overall conclusion was that changing the nature of the *N*-alkyl substituent of cathinone analogs has a profound influence on their actions; 4-MEC (**54**) is a SERT releasing agent/DAT blocker, whereas 4-MePPP (**55**) is a selective DAT blocker [54].

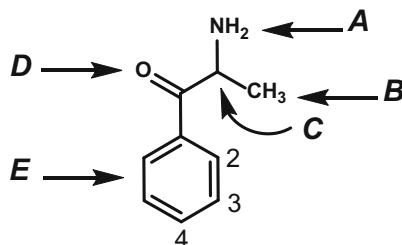
## 5 Overall Conclusions

From a practical perspective, the results of SAR and QSAR studies are typically used to investigate mechanisms of drug action and to forecast the action(s)/potency of novel agents. With respect to synthetic cathinones, SAR studies, and certainly QSAR studies, are still in their infancy. Only in the last year or two have QSAR methods (e.g., Hansch analyses, homology modeling and docking studies, HINT analyses) been applied to these agents.

SAR studies with synthetic cathinones began in the late 1970s, following the discovery of cathinone (**1**) as the active constituent of *khat*, with the simple findings that cathinone (**1**) was more potent than (+)cathine (**2**) as a central stimulant, that *S* (–)cathinone was more potent than its *R*(+)–enantiomer, and of the identification (i.e., the “rediscovery”) of what is now termed methcathinone (**13**). Early SAR studies examined the behavioral effects (i.e., locomotor stimulation, discriminative stimulus properties) of cathinone analogs because their mechanism of action was unknown. Once it was shown that these agents might be producing their effects by acting as DAT releasing agents, attention slowly shifted focus. Some agents also displayed releasing action at NET and/or SERT. Subsequently, MDPV (**27**) was identified as a drug of abuse that acted primarily as a reuptake inhibitor at DAT.

Substituents on the phenylpropanamine scaffold of cathinone can influence these actions. Figure 12 summarizes some of structural aspects of cathinone analogs that have been investigated in SAR studies, with an emphasis on DAT.

**Fig. 12** The structure of cathinone and the various structural alterations (at positions A–E) that have been examined in SAR studies to be summarized below



#### Synopsis of some SAR findings on synthetic cathinones:

(1) As reuptake inhibitors at DAT, optimal potency and selectivity are associated with a tertiary amine (A, Fig. 12) and an extended  $\alpha$ -side chain (B); with an extended side chain, the amine can be tertiary, secondary, or even primary – although potency generally decreases in this same rank order. To date, the most potent DA reuptake inhibitors are specifically associated with a pyrrolidine moiety as A and a fairly large  $\alpha$  (i.e., B)-substituent (e.g., *n*-butyl, cyclohexyl). Stereochemistry at C has not been extensively investigated, but for MDPV (**27**) and pyrovalerone (**29**), the *S*-isomers are substantially (i.e., >100-fold) more potent than their *R*-enantiomers. The carbonyl group at D generally has minimal influence on stimulant or DAT action, and its elimination converts the cathinone analog (a phenylpropanonamine) to its amphetamine analog (a phenylisopropylamine, e.g., compare **27** and **33** in Fig. 8). However, reduction of the carbonyl group of pyrovalerone (**29**) to a hydroxyl group results in diastereomers that lack affinity for DAT, NET, and SERT [27]. Substitution on the aryl ring (i.e., E) has not been extensively examined; however, there are preliminary indications that certain substituents might influence potency. This requires further investigation.

Typically, substituents optimal for action as a DAT reuptake inhibitor decrease or abolish actions at SERT.

(2) As releasing agents at DAT, a primary amine seems optimal with a simple *N*-methyl secondary amine being nearly as potent, and sometimes slightly more potent, than the primary amine. The increased behavioral potency of *N*-methyl analogs of cathinones over their primary amine counterparts might be related to the slightly higher affinity of the latter for DAT (although few comparisons are available), their greater resistance to metabolism, and/or their enhanced ability to penetrate the blood-brain barrier due to their increased lipophilicity. As the size/bulk of the amine (i.e., A) substituent increases, potency as a releasing agent (and perhaps selectivity) decreases. Increasing bulk at the terminal amine shifts the action of a DAT releasing agent to a DAT reuptake inhibitor. An  $\alpha$ -methyl group at B would seem optimal; increasing the length of the substituent can also reverse action from a releasing agent to a reuptake inhibitor at DAT. When the  $\alpha$ -substituent is a methyl group, *S*-isomers (i.e., C) are typically more potent (or equipotent) at DAT and SERT than their *R*-enantiomers; however, DAT/SERT

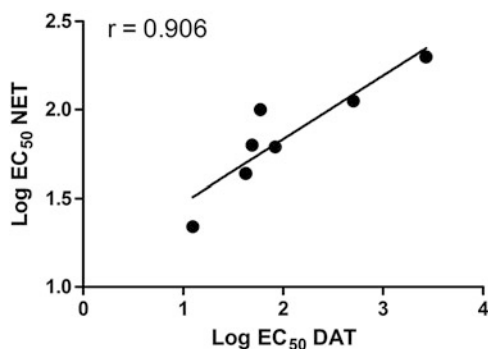
selectivity as governed by their *S/R* ratio can influence behavioral actions, and aryl substituents at *E* also play a role here. The carbonyl group at *D* plays a minimal role; however, reduction of the carbonyl group to a hydroxyl group (i.e., a phenylpropanolamine) reduces both its potency as a DAT substrate and as a centrally acting stimulant. Aryl substituents (i.e., *E*) at the ring 4-position generally decrease potency at DAT and can shift selectivity toward SERT; the larger the substituent, the greater the likelihood that it will favor SERT versus DAT. Substituents at the ring 2- and 3-positions have not been thoroughly investigated from an SAR perspective.

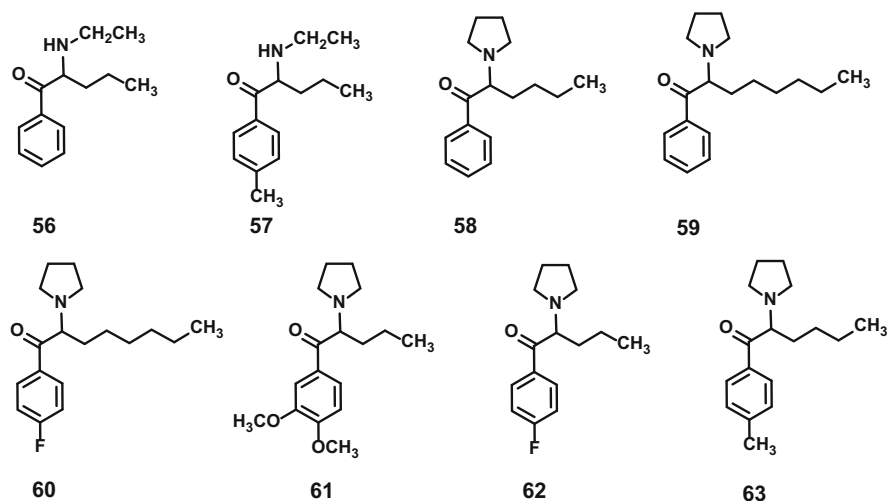
(3) Most SAR studies on synthetic cathinones have focused on DAT and SERT action; however, a role for the NET should not be overlooked. However, too few studies have been reported to allow for general SAR to be formulated. Nevertheless, for a series of seven synthetic cathinones (i.e., those shown in Table 5), it was found that their potency to act as substrates at DAT and NET was significantly ( $r = 0.906$ ) correlated (Fig. 13) [48]. Although SAR might not be identical at DAT and NET, there appear to be some similarities; additional agents will need to be examined.

The time has finally arrived where multiple agents are being investigated in the same study, under similar conditions, to allow reasonable SAR/QSAR conclusions to be formulated. It should be appreciated that the emerging SAR/QSAR results described here are based on a limited number of agents and investigations. At almost any time, novel agents might appear that will question or challenge these relationships. Nevertheless, the advent of novel agents will only strengthen and refine the current SAR.

Within the past several months, several new synthetic cathinones have been confiscated, or purchased from Internet sources, for purpose of physicochemical and spectral characterization (e.g., [55, 56]). These agents include  $\alpha$ -EAPP (56), 4-MeEAPP (57),  $\alpha$ -PHP (58),  $\alpha$ -POP (59), 4-fluoro-PV-9 (60), 3,4-dimethoxy- $\alpha$ -PVP (61), 4-fluoro- $\alpha$ -PVP (62), and MPHP (63) (Fig. 14).

**Fig. 13** Relationship between potencies of seven 4-substituted cathinone analogs (i.e., those in Table 6) to act as releasing agents at DAT and NET (from [48])





**Fig. 14** Structures of some novel synthetic cathinones that have not been thoroughly investigated

These agents (Fig. 14) have yet to be pharmacologically evaluated. However, it can be seen that they represent variations of common structural themes discussed herein. On the basis of the SAR reviewed above, it should now be possible to make some educated guesses as to the actions and approximate potencies of these novel substances.

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