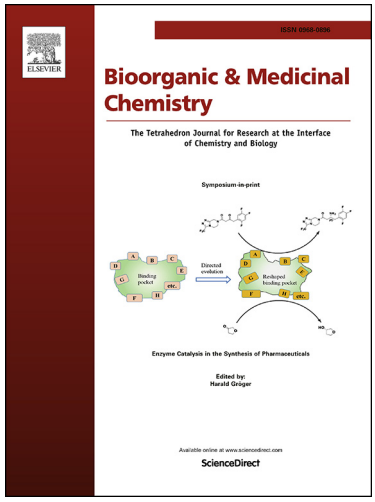
# Accepted Manuscript

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PII: S0968-0896(18)32129-1
DOI: https://doi.org/10.1016/j.bmc.2019.02.010
Reference: BMC 14746

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 21 December 2018
Revised Date: 4 February 2019
Accepted Date: 4 February 2019

Please cite this article as: Dimitrov, I.V., Harvey, M.G., Voss, L.J., Sleigh, J.W., Bickerdike, M.J., Denny, W.A., Ketamine esters and amides as short-acting anaesthetics: structure-activity relationships for the side-chain, *Bioorganic & Medicinal Chemistry* (2019), doi: https://doi.org/10.1016/j.bmc.2019.02.010

# Ketamine esters and amides as short-acting anaesthetics: structure-activity relationships for the side-chain

Ivaylo V. Dimitrov[a], Martyn G. Harvey[b], Logan J. Voss[b], James W. Sleigh[b], Michael J. Bickerdike[c], William A. Denny[a]

[a]*Auckland Cancer Society Research Centre, School of Medical Sciences, University of Auckland, New Zealand,* [b]*Waikato Clinical School, University of Auckland, New Zealand* [c]*Kea Therapeutics Ltd, Auckland, New Zealand.*

**Abstract**

*N*-Aliphatic ester analogues of the non-opioid ketamine (**1**) retain effective anaesthetic/analgesic properties while minimising ketamine's psychomimetic side-effects. We show that the anaesthetic/analgesic properties of these ester analogues depend critically on the length (from 2-4 carbons), polarity and steric cross-section of the aliphatic linker chain. More stable amide and ethylsulfone analogues generally showed weaker anaesthetic/analgesic activity. There was no correlation between the anaesthetic/analgesic properties of the compounds and their binding affinities for the *N*-methyl-*D*-aspartate (NMDA) receptor.

**Key words**

Ketamine; esters; anaesthesia; short-acting; structure-activity relationship

**Abbreviations**

DCC, *N*,*N*'-Dicyclohexylcarbodiimide; DCM, dichloromethane; DMAP, 4-Dimethylaminopyridine; LDA, Lithium diisopropylamide; LRR, loss of righting reflex; NMDA, *N*-methyl-*D*-aspartate; PWR, pedal withdrawal reflex score; TFAA, trifluoroacetic acid anhydride.

**Corresponding Author**

Ph (+649) 923 6156

Email i.dimitrov@auckland.ac.nz (I. V. Dimitrov)

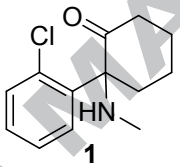
## 1. Introduction

Ketamine is a dissociative anaesthetic, which finds widespread application in the treatment of battlefield injuries, emergency departments and for use in children. Ketamine's lack of cardiovascular depression associated with other anaesthetics (especially opioids) offers significant advantages. It is however plagued by the feeling of detachment from the environment and the user and the near-death experiences it produces on recovery. It can also cause hallucinations. These side effects have limited its potential use. In recent times, however ketamine has enjoyed a resurgence due in large part to the impact it has had in

treating chronic depression and the rapid onset of its antidepressant effect. This in turn has led to increase in ketamine related publications in recent years. There has been a number of ketamine related analogues synthesised over the years, they have all proved less potent than the parent compound, however reduction of the hallucinogenic side effects have been achieved in certain cases.[1]

We have previously shown in a rat intravenous infusion model that aliphatic ester analogues of the non-opioid anaesthetic/analgesic ketamine (**1**) retain these desirable therapeutic activities,[2] while at the same time minimising its psychomimetic side-effects during recovery by undergoing very rapid metabolism to the much more polar and inactive acids,[3] resulting in a 10-fold shorter period for recovery compared with that of **1**. Structure-activity relationships for these alkyl-linked esters $N(CH_2)_nCO_2R$ (e.g., compounds **4**, **6**) showed that their potency as sedatives was not very dependent upon the *N*-alkylester chain length (from 2-4 carbons), but dropped away more quickly with the size of the ester R group.

In the present paper we extend these structure-activity relationships for the side chain by further variations (Table 1) to the length and nature of the spacer alkyl chain and to the nature of the terminating group.




**1**

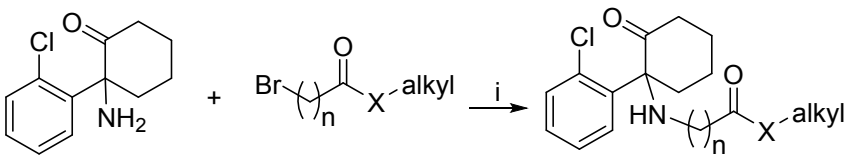
*ketamine*

## 2. Results and discussion

### 2.1. Chemistry

The compounds of Table 1 were prepared from racemic norketamine[4] (**23**), by reaction with the appropriate alkyl bromides in acetonitrile under reflux, in the presence of $K_2CO_3$ and KI, for the appropriate time (between 12-168 h) (Scheme 1). The methylaluminium amide and dimethylaluminium amide reagents[5] for the preparation of amides **19** and **20** were generated by reacting trimethylaluminium with methylamine or dimethylamine hydrochloride at room temperature for an hour. Subsequent addition of the known[2] norketamine ester **24** and reflux overnight afforded the desired amides (Scheme 2).

**Scheme 1. General synthesis of the compounds of Table 1**

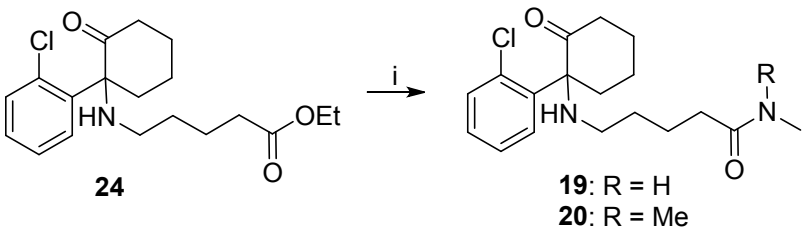



**23**

X = O, NH

*Reagents and conditions*: (i) alkyl bromide, $K_2CO_3$, KI, MeCN, heat (4 -168 h)

**Scheme 2. Synthesis of compounds 19, 20 of Table 1**

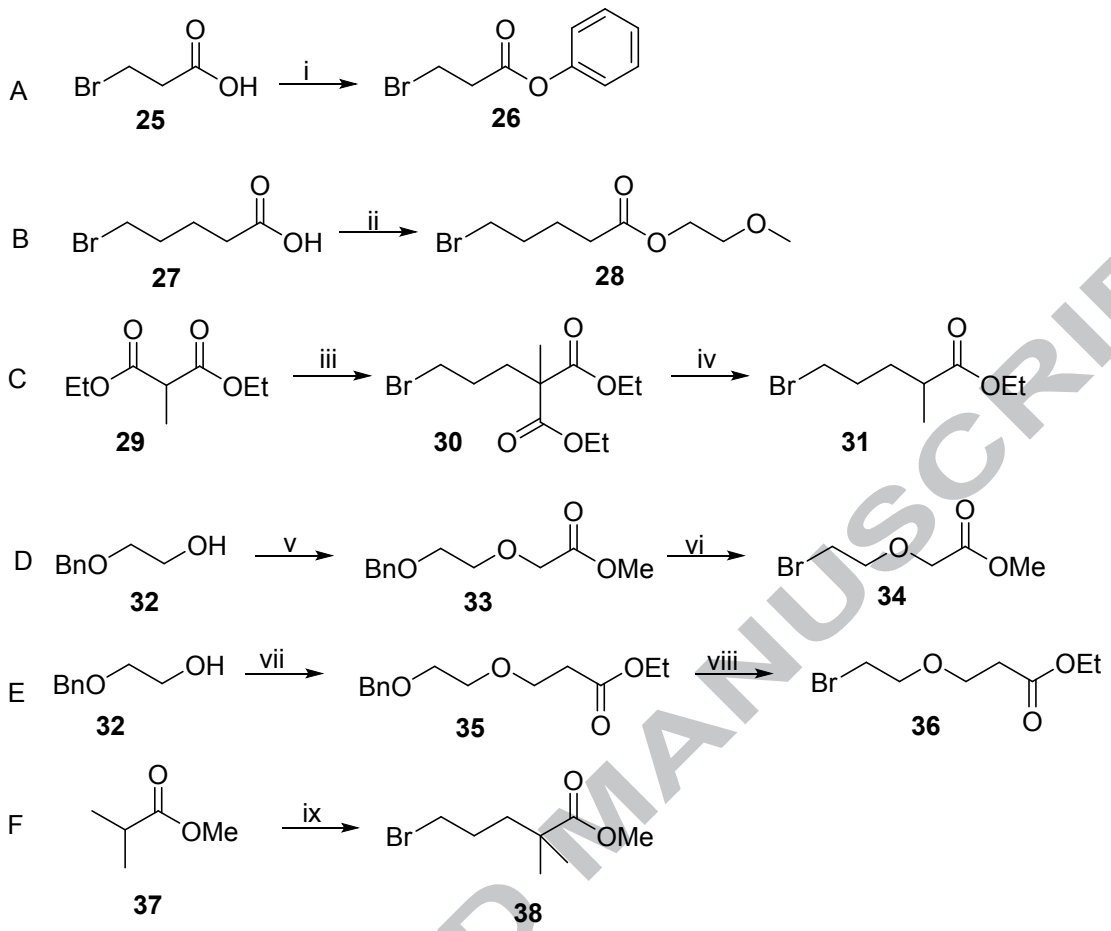



*Reagents and conditions*: (i) Al reagent, toluene, reflux, 15 h, 72% for **19** and 71% for **20**

Many of the required alkyl bromides were commercially available or prepared by literature methods; others were prepared by the routes shown in Scheme 3. Phenyl 3-bromopropanoate (**26**) for the preparation of **5** was prepared[6] by reaction of 3-bromopropionic acid (**25**) with phenol in the presence of TFAA (Scheme 3A). Methoxy ethyl 5-bromopentanoate (**28**) for the preparation of **12** was prepared by reacting 5-bromovaleric acid (**27**) with 2-methoxyethanol in the presence of DCC and DMAP (Scheme 3B). Ethyl 5-bromo-2-methylpentanoate (**31**) for the preparation of **9** was prepared by reacting diethyl methylmalonate (**29**) with 1,3-dibromopropane in the presence of NaH to give diester (**30**), which was then treated with conc. HBr under reflux (Scheme 3C).[6] Similarly methyl 5-bromo-2,2-dimethylpentanoate (**38**) was prepared by reacting methyl isobutyrate (**37**) with 1,3-dibromopropane in the presence of LDA (Scheme 3F).

Methyl 2-(2-bromoethoxy)acetate (**34**) for the preparation of **11** was synthesized by reacting 2-(benzyloxy)ethanol (**32**) with methyl bromoacetate in the presence of NaH to yield ether **33**. Benzyl deprotection of this by hydrogenation (Pd/C) gave an alcohol, which in turn underwent an Appel reaction[7] ($CBr_4/PPh_3$) to give **34** (Scheme 3D). Ethyl 3-(2-bromoethoxy)propanoate (**36**) for the preparation of **13** was synthesized by similarly reacting **32** with ethyl acrylate in the presence of Na metal to give ether **35**. Benzyl group deprotection ($Pd/C/H_2$) of this ether yielded an alcohol, which in turn underwent an Appel reaction to give **36** (Scheme 3E).[8]

**Scheme 3. Preparation of required bromides**

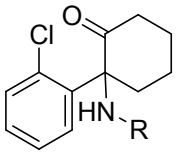



*Reagents and conditions*: (i) phenol, TFAA, 25°C, 12 h, 74%; (ii) 2-methoxyethanol, DCC, DMAP, DCM, 25 °C, 48 h, 84%; (iii) NaH, 1,3-dibromopropane, THF, 0-15 °C, 20 h, 87%; (iv) HBr (48%), reflux, 7 h, then 25 °C. 15 h, 80%; (v) methyl bromoacetate, NaH, THF, 0-25 °C, 12 h, 55%; (vi) Pd/C, $H_2$, EtOAc, 12 h, then $CBr_4$, $PPh_3$, THF, 25 °C, 3 h, 62% over 2 steps; (vii) ethyl acrylate, Na, THF, 25 °C, 20 h, 60%; (viii) Pd/C, $H_2$, EtOAc, 12 h, then $CBr_4$, $PPh_3$, THF, 25 °C, 3 h, 64% over 2 steps; (ix) 1,3-dibromopropane, LDA, THF, -78-0 °C, 4 h, 86%.

## 2.2. Biology

Table 1 gives both structural and biological data for ketamine (**1**) itself and two previously-reported esters[2,3] (**4**, **6**), along with 20 new analogues. Compounds **2** and **3** are examples of the shortest possible (1-atom) chain, and both were inactive at the highest dose tested (200 mg/kg). This contrasts with the results for the previously-reported[2] $(CH_2)_2$- and $(CH_2)_4$-linked compounds **4** and **6**, which had analgesic potencies close to that of **1**, and defines two atoms as the minimum length spacer. There is a marked drop in calculated pKa in going from a $(CH_2)_2$ to a $CH_2$ link (from 4.35 to 3.17) but it is not known if this is the reason for the loss of activity. Compounds **7** and **8** explore longer chain lengths (5 and 6 atoms respectively). Both were inactive, suggesting an optimal upper size limit to the spacer chain as well.

**Table 1. Structural and biological data for new ketamine ester and amide analogues**




| N | n | R | LORR[a] mg/kg | PWR[b] mg/kg | RORR[c] sec | NMDA[d] $IC_{50}$ (μM) | pKa[e] |
|---|---|---|---|---|---|---|---|
| **1**[f] | | | 23 | 28 | 1075 | 0.7 | 7.49 |
| **Ester chain length** | | | | | | | |
| **2** | 1 | $CH_2CO_2Me$ | >200 | >200 | NA[g] | 308 | 3.17 |
| **3** | 1 | $CH_2CO_2{}^iPr$ | >200 | >200 | NA | 45 | 3.67 |
| **4**[f] | 2 | $(CH_2)_2CO_2{}^iPr$ | 33 | 37 | 83 | 134 | 4.35 |
| **5** | 2 | $(CH_2)_2CO_2Ph$ | >200 | >200 | NA | 73 | 5.02 |
| **6**[f] | 4 | $(CH_2)_4CO_2Me$ | 34 | 44 | 99 | 3 | 6.29 |
| **7** | 5 | $(CH_2)_5CO_2Me$ | >200 | >200 | NA | 44 | 6.47 |
| **8** | 6 | $(CH_2)_6CO_2Me$ | >200 | >200 | NA | 51 | 6.57 |
| **Ester chain hindrance** | | | | | | | |
| **9** | 4 | $(CH_2)_3CHMeCO_2Et$ | 50 | 55 | 260 | 107 | 6.42 |
| **10** | 4 | $(CH_2)_3C(Me)_2CO_2Me$ | 52 | >200 | 580 | 125 | 6.36 |
| **Ester chain polarity** | | | | | | | |
| **11** | 4 | $(CH_2)_2OCH_2CO_2Me$ | >200 | >200 | NA | 13 | 5.03 |
| **12** | 4 | $(CH_2)_4CO_2(CH_2)_2OMe$ | >200 | >200 | NA | 19 | 6.38 |
| **13** | 5 | $(CH_2)_2O(CH_2)_2CO_2Et$ | >200 | >200 | NA | 87 | 5.34 |
| **Ester replacement** | | | | | | | |
| **14** | 2 | $(CH_2)_2CONH_2$ | >200 | >200 | NA | 500 | 5.45 |
| **15** | 2 | $(CH_2)_2CONHMe$ | 58 | >200 | 85 | 73 | 5.14 |
| **16** | 2 | $(CH_2)_2CONHEt$ | 110 | 113 | 623 | 416 | 5.42 |
| **17** | 2 | $(CH_2)_2CONH{}^iPr$ | 112 | 139 | 237 | 935 | 5.33 |
| **18** | 2 | $(CH_2)_2CONMe_2$ | 82 | >200 | 82 | 58 | 5.45 |
| **19** | 4 | $(CH_2)_4CONHMe$ | 83 | >200 | 125 | 77 | 6.37 |
| **20** | 4 | $(CH_2)_4CONMe_2$ | >200 | >200 | NA | >1000 | 6.42 |
| **21** | 4 | $(CH_2)_4CONpiperidine$ | 67 | 130 | 556 | 41 | 9.56 |
| **22** | 2 | $(CH_2)_2SO_2Et$ | >200 | >200 | NA | >1000 | 4.20 |

Footnotes for Table 1

[a]LORR: minimal dose for loss of righting reflex; [b]PWR: minimal dose for loss of pedal withdrawal reflex; [c]RORR: time for return of righting reflex after infusion stopped; [d]NMDA: Binding affinity ($IC_{50}$) at the NMDA glutamate ion channel using [$^3$H]MK-801 as the competitive radioligand (see text for details); [e]pKa of amine, calculated using ACD/PhysChem Suite v12; [f]Data from ref. 1; [g]NA: not attained.

Compounds **9** and **10** are methyl-substituted analogues of **6** and explore the effect of steric bulk around the ester. Both compounds had slightly lower sedative potency than **6**, but longer

recovery times (roughly 2.5 and 6 times than that of **6**), consistent with increasing resistance to enzymic hydrolysis expected with greater steric bulk close to the ester.[9]

Compounds **11-13** investigate the effect of inserting a polar ether link in the side chain (**11**,**13**) or in the ester group (**12**) of compounds that are essentially analogues of **6** (4-atom linker chain). Replacement of $CH_2$ by an O atom lowers logP by only about half a unit (calculated) but irrespective of its placement abolishes the excellent sedative/analgesic properties of **6**.

Compounds **14-21** explore the replacement of the ester unit with similar but more stable amide groups). Of the five $(CH_2)_2$-linked amides (**14-18**), the primary amide **14** did not show activity due to insolubilisation. The other four secondary/tertiary amides (**15-18**) showed sedative activity, but were 2-3 fold less potent than ester **6**. Comparisons between esters **4** and **6** and the direct corresponding amides **17** and **19** showed the amides to be anaesthetic and analgesic, but with lesser potency and longer offsets than their counterpart esters. Interestingly, while the $(CH_2)_4$-linked tertiary amide **20** was inactive, the corresponding piperidide **21** showed significant anaesthetic potency, but with a long offset (556 sec). Finally, compound **22** explored the use of a sulfone as a stable ester analogue but this very polar compound was completely inactive.

In Table 1 we also present the binding affinities of these compounds for the *N*-methyl-*D*-aspartate (NMDA) glutamate ion channel, determined by a radioligand binding assay using Wistar rat brain preparations.[10] Many of the pharmacological effects of ketamine (anaesthetic, analgesic, psychomimetic, anti-depressant), have previously been linked to this property[13,14] since ketamine has high binding affinity for the NMDA receptor, with its Ki reported in the literature to be 1.9 μM.[15] However, our data clearly suggests that NMDA potency does not correlate at all with the anaesthetic or analgesic activity of the compounds discussed here. While ketamine (**1**) had a minimal dose for loss of righting reflex of 23 mg/kg and an $IC_{50}$ for NMDA inhibition of 0.7 nM, the most active anaesthetic analogues in Table 1 (compounds **4**, **6**, **9**, **10**) had NMDA affinities ranging from 3-580 nM; the same range as that for the most inactive compounds **2**, **5**, **7**, **8**, **11-14** (i.e. 13-560 nM).

**2.3. Conclusions**

Compounds **2-8** of Table 1 define the acceptable length of the *N*-ester spacer chain to be from 4-6 atoms. Compounds **9** and **10** suggest that more bulk around the carbonyl carbon does result in slower hydrolysis (longer recovery times) but does not markedly affect anaesthetic activity. In contrast, compounds **11** and **13** show that polarity in the spacer chain abolishes activity, even when the chain is in the allowable length range. Compounds **14-21** explore the use of more stable aliphatic amide groups; the bulk of these were active but were considerably less potent, with the best being the piperidide **21**. There was no relationship between anaesthetic/antinociceptive activity and NMDA channel affinity for these compounds.

## 3. Experimental

### 3.1. Chemistry

All reagents and solvents were obtained from commercial suppliers and used without further purification unless otherwise stated. Reactions requiring anhydrous conditions were performed under nitrogen atmospheres. Reactions were monitored by thin layer chromatography (TLC) on preloaded silica gel F254 plates (Sigma-Aldrich) with a UV indicator. Column chromatography was performed with Merck 230-400 mesh silica gel. $^{1}H$ and $^{13}C$ NMR spectra were obtained with a Bruker Avance 400 spectrometer at 400 MHz for $^{1}H$ and 101 MHz for $^{13}C$ spectra. Spectra were obtained in $CDCl_3$ or $(CD_3)_2SO$. The chemical shifts are reported in parts per million (δ) downfield using tetramethylsilane ($SiMe_4$) as internal standard. Spin multiplicities are given as s (singlet), d (doublet), dd (double doublet), br (broad), m (multiplet), and q (quartet). Coupling constants (*J* values) were measured in hertz (Hz). All LC/MS data were gathered by direct injection of methanolic solutions into a Surveyor MSQ mass spectrometer using an atmospheric pressure chemical ionization (APCI) with a corona voltage of 50 V and a source temperature of 400 $^{o}$C. High-resolution electrospray ionization (HRESIMS) mass spectra were determined on a Bruker micrOTOFQ II mass spectrometer. Final products were analyzed by reverse-phase HPLC (Alltima C18 5 μm column, 150 mm × 3.2 mm; Alltech Associated, Inc., Deerfield, IL) using an Agilent HP1100 equipped with a diode array detector. The mobile phase was 80% MeCN/20% $H_2O$ (v/v) in 45 mM $HCO_2NH_4$ at pH 3.5 and 0.5 mL/min. The purity was determined by monitoring at 272 nm and was ≥95% for final products unless otherwise stated. DCM refers to dichloromethane, DMF refers to *N,N*-dimethylformamide, EtOAc refers to ethyl acetate, EtOH refers to ethanol.

**3.1.1. Methyl (1-(2-chlorophenyl)-2-oxocyclohexyl)glycinate hydrochloride (2) (Scheme 1).** A solution of racemic norketamine (**23**) (0.4 g, 1.78 mmol), methyl 2-bromoacetate (0.32 g, 2.14 mmol), KI (0.089g, 0.54 mmol), $K_2CO_3$ (0.74 g, 5.35 mmol) in MeCN (10 mL) was heated to a 100 $^{o}$C in sealed tube for 12 h. The reaction mixture was cooled to room temperature, filtered and the solvent evaporated. The residue was purified by column chromatography on silica gel eluting with EtOAc/hexanes (0-35%) to give **2** (0.42 g, 80%) as pale yellow oil. This was dissolved in $Et_2O$ (5 mL), cooled to 0 $^{o}$C, and HCl in $Et_2O$ (2M, 2.11 mmol) was added dropwise. The solvent was evaporated under reduced pressure, the residue was taken up in EtOAc (1 mL) and sonicated at room temperature for 2 min. The white precipitate was diluted with EtOAc (5 mL), filtered washed with EtOAc and dried under vacuum to give **2**; $^{1}$HNMR ($CDCl_3$) δ 7.55-7.52 (dd, *J*= 7.84 Hz, 1.72 Hz, 1H), 7.38-7.35 (dd, *J*= 7.76 Hz, 1.48 Hz, 1H), 7.33-7.28 (td, *J*= 7.44 Hz, 1.52 Hz, 1H), 7.26-7.21 (td, *J*= 9.36 Hz, 1.76 Hz, 1H), 3.66 (s, 3H), 3.33-3.28 (d *J*=17.64 Hz, 1H), 3.15-3.11 (d, d *J*=17.65 Hz, 1H), 2.74 (br. 1H), 2.66-2.58 (m, 2H), 2.49-2.45 (m, 1H), 2.01-1.74 (m 5H); $^{13}C$ ($CDCl_3$) δ 208.20, 172.88, 138.65, 133.58, 131.32, 129.04, 128.97, 127.00, 69.04, 51.99, 44.76, 39.28, 39.25, 26.95, 21.66; MS *m/z* 296.20 ($MH^+$). Calculated for $C_{15}H_{19}ClNO_3$ ($MH^+$) 296.1048, found 296.1045.

**3.1.2. Isopropyl (1-(2-chlorophenyl)-2-oxocyclohexyl)glycinate hydrochloride (3)**. Similar reaction of **23** (0.2 g, 0.89 mmol) and isopropyl 2-bromoacetate (2.67 g, 14.9 mmol), KI (0.05 g, 0.31 mmol) $K_2CO_3$ (0.43 g, 3.13 mmol) in MeCN (7 mL) at 120 °C in a sealed tube for 168 h, followed by chromatographic purification on silica gel, eluting with EtOAc/hexanes (0-15%), gave a pale yellow oil (0.08 g, 27%), which was converted to **3** with HCl in $Et_2O$ as above. $^1$HNMR ($CDCl_3$) δ 7.55-7.5 2 (dd, *J*= 7.84 Hz, 1.72 Hz, 1H), 7.38-7.35 (dd, *J*= 7.76 Hz, 1.44 Hz, 1H), 7.33-7.28 (td, *J*= 7.44 Hz, 1.48 Hz, 1H), 7.26-7.21 (td, *J*= 9.36 Hz, 1.76 Hz, 1H), 5.14-5.05 (q, *J*=6.28 Hz, 1H), 3.29-3.24 (d, *J*=17.69 Hz, 1H), 3.11-3.07 (d, *J*=17.69 Hz, 1H), 2.67-2.56 (m, 2H), 2.52-2.48 (m, 1H), 1.99-1.77 (m, 5H), 1.21-1.20 (d, *J*=6.16 Hz, 6H); $^{13}$C ($CDCl_3$) δ 208.25, 172.19, 139.09, 133.62, 131.40, 129.12, 128.96, 127.05, 68.97, 68.57, 45.26, 39.37, 39.29, 26.81, 22.01, 21.96; MS *m/z* 324.20 ($MH^+$) Calculated for $C_{17}H_{22}ClNO_3$ ($MH^+$) 324.1362, found 324.1362.

**3.1.3. Phenyl 3-((1-(2-chlorophenyl)-2-oxocyclohexyl)amino)propanoate hydrochloride (5)**. Similar reaction of **23** (0.3 g, 1.34 mmol) and phenyl 3-bromopropanoate (**26**) (1.84 g, 8.07 mmol) with $K_2CO_3$/KI in MeCN at 100 °C for 48 h, followed by chromatography on silica gel (EtOAc/hexanes) gave a pale yellow oil (0.35 g, 70%), which was converted to **5** as above. $^1$HNMR ($CDCl_3$) δ 7.61-7.58 (dd, *J*= 7.6 Hz, 1.6 Hz, 1H), 7.40-7.36 (m, 3 H), 7.33-7.29 (td, *J*= 9.2 Hz, 1.6 Hz, 1H), 7.26-7.21 (m, 3H), 7.09-7.07 (m, 2H), 2.75-2.64 (m, 4 H), 2.5-2.47 (m, 3H), 2.0-1.9 (m 2 H), 1.79-1.57 (m, 3H); $^{13}$C ($CDCl_3$) δ 208.33, 171.81, 150.74, 138.67, 133.82, 131.43, 129.66, 129.18, 128.89, 126.98, 126.12, 121.76, 69.77, 39.58, 39.19, 38.26, 35.40, 27.36, 21.91; MS *m/z* 372.10 ($MH^+$) Calculated for $C_{21}H_{23}ClNO_3$ ($MH^+$) 372.1361, found 372.1352.

**3.1.4. Methyl 5-((1-(2-chlorophenyl)-2-oxocyclohexyl)amino)hexanoate hydrochloride (7).** Similar reaction of **23** (0.35 g, 1.56 mmol) and methyl 6-bromohexanoate (0.49 g, 2.4 mmol) with $K_2CO_3$/KI in MeCN at 120 °C for 12 h, gave a pale yellow oil (0.40 g, 73%), which was purified and converted to **7** as above. $^1$HNMR ($CDCl_3$) δ 7.55-7.52 (dd, *J*= 7.84 Hz, 1.72 Hz, 1H), 7.38-7.35 (dd, *J*= 7.76 Hz, 1.48 Hz, 1H), 7.33-7.28 (td, *J*= 7.44 Hz, 1.52 Hz, 1H), 7.26-7.21 (td, *J*= 9.36 Hz, 1.76 Hz, 1H), 3.65 (s, 3H), 2.75-2.71 (m, 1H), 2.53-2.43 (m 2H), 2.28-2.25 (t, *J*= 7.36 Hz, 2H), 2.06-1.98 (m, 3H), 1.81-1.76 (m, 3H), 1.65-1.39 (m, 5H), 1.39-1.13 (m, 3H); $^{13}$C ($CDCl_3$) δ 209.09, 174.31, 138.69, 133.83, 131.32, 129.35, 128.76, 126.82, 70.01, 51.62, 42.28, 39.64, 39.22, 34.13, 30.45, 27.97, 26.87, 24.91 22.03; MS *m/z* 352.20 ($MH^+$) Calculated for $C_{19}H_{27}ClNO_3$ ($MH^+$) 352.1674, found 352.1686

**3.1.5. Ethyl 7-((1-(2-chlorophenyl)-2-oxocyclohexyl)amino)heptanoate hydrochloride (8).** Similar reaction of **23** (0.35 g, 1.56 mmol) and ethyl 7-bromoheptanoate (0.56 g, 2.4 mmol) with $K_2CO_3$/KI in MeCN at 90 °C for 48 h gave a pale yellow oil (0.55 g, 91%) which was purified and converted to **8** as above. $^1$HNMR ($CDCl_3$) δ 7.55-7.52 (dd, *J*= 7.84 Hz, 1.72 Hz, 1H), 7.38-7.35 (dd, *J*= 7.76 Hz, 1.48 Hz, 1H), 7.33-7.28 (td, *J*= 7.44 Hz, 1.52 Hz, 1H), 7.26-7.21 (td, *J*= 9.36 Hz, 1.76 Hz, 1H), 4.14-4.08 (m, 2H), 2.76-2.67 (M, 1H), 2.52-2.41 (m, 2H), 2.34-2.28 (m, 1H), 2.27-2.23 (t, *J*=7.64 Hz, 2H), 2.05-1.35 (m, 14 H), 1.25-1.22 (t, *J*=7.16 Hz, 3H); $^{13}$C ($CDCl_3$) δ 208.99, 173.98, 138.83, 133.82, 131.32, 129.36, 128.74,

126.80, 69.98, 60.58, 42.45, 39.63, 39.18, 34.47, 30.63, 29.14, 27.87, 27.02, 25.06, 22.04, 14.38; MS *m/z* 380.20 ($MH^+$) Calculated for $C_{21}H_{31}ClNO_3$ ($MH^+$) 380.1987, found 380.1919

**3.1.6. Ethyl 5-((1-(2-chlorophenyl)-2-oxocyclohexyl)amino)-2-methylpentanoate hydrochloride (9).** Similar reaction of **23** (0.35 g, 1.56 mmol), ethyl 5-bromo-2-methylpentanoate (**31**) (0.55 g, 2.4 mmol) with $K_2CO_3$/KI in MeCN at 95 °C for 12 h gave a pale yellow oil (0.45 g, 79 %), which was purified and converted to **9**as above. $^1$HNMR ($CDCl_3$) δ 7.55-7.52 (dd, *J*= 7.84 Hz, 1.72 Hz, 1H), 7.38-7.35 (dd, *J*= 7.76 Hz, 1.48 Hz, 1H), 7.33-7.28 (td, *J*= 7.44 Hz, 1.52 Hz, 1H), 7.26-7.21 (td, *J*= 9.36 Hz, 1.76 Hz, 1H), 4.13-4.07 (q, *J*=7.12 Hz), 2.74-2.71 (m, 1), 2.54-2.29 (m, 4 H), 2.15-1.93 (m, 3H), 1.92-1.82 (m, 1H), 1.82-1.72 (m 3H), 1.68-1.37 (m, 3H), 1.28-1.19 (t, *J*= 7.08 Hz, 3H), 1.12-0.98 (d, *J*= 3.96 Hz, 3H); $^{13}$C ($CDCl_3$) δ 208.94, 176.80, 138.65, 133.76, 131.27, 129.28, 128.72, 126.76, 69.94, 60.26, 42.31, 39.57, 39.48 39.44, 39.18, 34.74, 31.37, 28.47, 22.15, 17.20; MS *m/z* 366.20 ($MH^+$) Calculated for $C_{20}H_{29}ClNO_3$ ($MH^+$) 366.1830, found 366.1827

**3.1.7. Ethyl 5-((1-(2-chlorophenyl)-2-oxocyclohexyl)amino)-2,2-dimethylpentanoate hydrochloride (10).** Similar reaction of **23** (0.35 g, 1.56 mmol) and methyl 5-bromo-2,2-dimethylpentanoate (**38**) (0.55 g, 2.4 mmol) with $K_2CO_3$/KI in MeCN at 95 °C for 12 h gave a pale yellow oil (0.49 g, 86 %), which was purified and converted to **10** as above. $^1$HNMR ($CDCl_3$) δ 7.55-7.52 (dd, *J*= 7.84 Hz, 1.72 Hz, 1H), 7.38-7.35 (dd, *J*= 7.76 Hz, 1.48 Hz, 1H), 7.33-7.28 (td, *J*= 7.44 Hz, 1.52 Hz, 1H), 7.26-7.21 (td, *J*= 9.36 Hz, 1.76 Hz, 1H), 3.64 (s, 3H), 2.79-2.69 (m, 1H), 2.56-2.40 (m. 2H), 2.32-2.22 (m 1H), 2.04-1.92 (m, 3H), 1.91-1.82 (m, 1H), 1.82-1.70 (m, 3H), 1.52-1.44 (m, 2H), 1.42-1.32 (m, 1H), 1.14 (s, 6H); $^{13}$C ($CDCl_3$) δ 209.16, 178.56, 138.66, 133.88, 131.36, 129.98, 129.37, 126.83, 70.05, 51.88, 43.05, 42.31, 39.68, 39.31, 38.39, 28.07, 26.44, 25.35, 22.06; MS *m/z* 366.20 ($MH^+$) Calculated for $C_{20}H_{29}ClNO_3$ ($MH^+$) 366.1830, found 366.1842

**3.1.8. Methyl 2-(2-((1-(2-chlorophenyl)-2-oxocyclohexyl)amino)ethoxy)acetate hydrochloride (11)**. Similar reaction of **23** (0.35 g, 1.56 mmol) and methyl 2-(2-bromoethoxy)acetate (**34**) (0.61 g, 3.1 mmol) with $K_2CO_3$/KI in MeCN at 110 °C for 48 h gave a pale yellow oil (0.37 g, 70 %), which was purified and converted to **11** as above. $^1$HNMR ($CDCl_3$) δ 7.55-7.52 (dd, *J*= 7.84 Hz, 1.72 Hz, 1H), 7.38-7.35 (dd, *J*= 7.76 Hz, 1.48 Hz, 1H), 7.33-7.28 (td, *J*= 7.44 Hz, 1.52 Hz, 1H), 7.26-7.21 (td, *J*= 9.36 Hz, 1.76 Hz, 1H), 4.10 (s, 2H), 3.75 (s, 3H), 3.64-3.60 (m, 2H), 2.72-2.62 (m, 1H), 2.60-2.43 (m, 3H), 2.40-2.31 (m, 1H), 2.20-1.88 (m, 2H), 1.88-1.82 (m, 3H); $^{13}$C ($CDCl_3$) δ 208.06, 171.11, 138.97, 133.61, 131.24, 129.23, 128.69, 126.8, 71.84, 69.35, 68.36, 51.94, 42.16 39.41, 39.06, 27.07, 21.71; MS *m/z* 340.10 ($MH^+$) Calculated for $C_{17}H_{23}ClNO_4$ ($MH^+$) 340.1310, found 340.1313

**3.1.9. 2-Methoxyethyl 5-((1-(2-chlorophenyl)-2-oxocyclohexyl)amino) pentanoate hydrochloride (12)**. Similar reaction of **23** (0.35 g, 1.56 mmol) and 2-methoxyethyl 5-bromopentanoate (**28**) (0.75 g, 3.1 mmol) with $K_2CO_3$/KI at 100 °C for 48 h gave a pale yellow oil (0.40 g, 67%), which was converted to **12** as above. $^1$HNMR ($CDCl_3$) δ 7.55-7.52 (dd, *J*= 7.84 Hz, 1.72 Hz, 1H), 7.38-7.35 (dd, *J*= 7.76 Hz, 1.48 Hz, 1H), 7.33-7.28 (td, *J*= 7.44 Hz, 1.52 Hz, 1H), 7.26-7.21 (td, *J*= 9.36 Hz, 1.76 Hz, 1H), 4.22-4.18 (m, 2H), 3.58-3.54

(m, 2H), 3.38 (s, 3H), 2.77-2.69 (m, 1H), 2.53-2.40 (m, 2H), 2.34-2.29 (m, 3H), 2.10-1.95 (m, 2H), 1.93-1.83 (m, 1H), 1.82-1.72 (m, 3H), 1.64-1.60 (m, 2H), 1.5-1.40 (m, 2H); $^{13}$C ($CDCl_3$) δ 209.09, 173.77, 138.68, 133.85, 131.36, 129.34, 128.80, 126.84, 70.66, 70.01, 63.50, 59.17, 42.11, 39.64, 39.26, 34.08, 30.28, 27.99, 22.72, 22.04; MS *m/z* 382.20 ($MH^+$) Calculated for $C_{20}H_{29}ClNO_4$ ($MH^+$) 382.1780, found 382.1774

**3.1.10. Ethyl 3-(2-((1-(2-chlorophenyl)-2-oxocyclohexyl)amino)ethoxy)propanoate hydrochloride (13).** Similar reaction of **23** (0.18 g, 0.81 mmol) and ethyl 3-(2-bromoethoxy)propanoate (**36**) (0.36 g, 1.61 mmol) with $K_2CO_3$/KI in MeCN at 100 °C for 20 h gave a pale yellow oil (0.17 g, 60 %), which was purified and converted to **13** as above. $^1$HNMR ($CDCl_3$) δ 7.55-7.52 (dd, *J*= 7.84 Hz, 1.72 Hz, 1H), 7.38-7.35 (dd, *J*= 7.76 Hz, 1.48 Hz, 1H), 7.33-7.28 (td, *J*= 7.44 Hz, 1.52 Hz, 1H), 7.26-7.21 (td, *J*= 9.36 Hz, 1.76 Hz, 1H), 4.19-4.11 (1, *J*=7.16 Hz, 2H), 3.74-3.66 (m, 2H), 3.60-3.46 (m, 2H), 2.69-2.42 (m, 6H), 2.34-2.26 (m, 1H), 2.2-1.88 (m, 2H), 1.84-1.72 (m, 3H), 1.29-1.23 (t, *J*=7.13 Hz, 3H); $^{13}$C ($CDCl_3$) δ 207.94, 171.81, 139.19, 133.64, 131.28, 129.24, 128.68, 126.84, 70.76, 69.31, 66.30, 60.68, 42.09, 39.44, 28.98, 35.29, 26.97, 21.73, 14.41; MS *m/z* 368.10 ($MH^+$) Calculated for $C_{19}H_{27}ClNO_4$ ($MH^+$) 368.1623, found 368.1635.

**3.1.11. 3-((1-(2-Chlorophenyl)-2-oxocyclohexyl)amino) propanamide hydrochloride (14).** Similar reaction of **23** (0.30 g, 1.35 mmol) and 3-bromopropanamide (0.51 g, 3.33 mmol) with $K_2CO_3$/KI in MeCN at 120 °C for 168 h, followed by purification by column chromatography on silica gel, gave a pale yellow oil (0.20 g, 52%), which was converted to **14** as above. $^1$HNMR ($CDCl_3$) δ 8.24 (br, 1H), 7.40-7.25 (m, 2H), 6.81 (br, 2H), 6.27-6.20 (dd, *J*=10.16 Hz, 17.08 Hz, 1H), 6.07-6.02 (dd, *J*= 17.12Hz, 2.28 Hz, 1H), 3.31-3.26 (q, *J*=7.0 Hz, 2H), 2.27-2.23 (m, 4H), 2.21- 2.18 (m, 2H), 1.89-1.72 (m, 4H); $^{13}$C ($CDCl_3$) δ 205.16, 172.97, 140.02, 131.48, 130.48, 129.05, 128.43, 126.85, 124.78, 68.39, 56.47, 35.18, 34.89, 30.70, 25.01, 20.57; MS *m/z* 295.20 ($MH^+$) Calculated for $C_{15}H_{20}ClN_2O_2$ ($MH^+$) 295.1208, found 295.1210

**3.1.12. 3-((1-(2-Chlorophenyl)-2-oxocyclohexyl)amino)-*N*-methylpropanamide hydrochloride (15).** Similar reaction of **23** (0.30 g, 1.35 mmol) and 3-bromo-*N*-methylpropanamide (0.56 g, 3.34 mmol) with $K_2CO_3$/KI in MeCN at 120 °C for 168 h, followed by purification as above, gave a pale yellow oil, gave a pale yellow oil (0.20 g, 50%) which was converted to **15** as above. $^1$HNMR ($CDCl_3$) δ 7.55-7.52 (dd, *J*= 7.84 Hz, 1.72 Hz, 1H), 7.38-7.35 (dd, *J*= 7.76 Hz, 1.48 Hz, 1H), 7.33-7.28 (td, *J*= 7.44 Hz, 1.52 Hz, 1H), 7.26-7.21 (td, *J*= 9.36 Hz, 1.76 Hz, 1H), 3.01-2.92 (m, 1H), 2.69-2.62 (d, *J*=4.81 Hz, 3H), 2.52-2.46 (m, 2H), 2.36-2.26 (m, 3H), 2.10-1.90 (m 4H), 1.84-1.72 (m 2H); $^{13}$C ($CDCl_3$) δ 210.84, 173.30, 136.99, 134.20, 131.64, 129.69, 129.50, 127.16, 70.46, 39.83, 39.55, 38.03, 36.09, 29.72, 26.02, 22.33; MS *m/z* 309.20 ($MH^+$) Calculated for $C_{16}H_{22}ClN_2O_2$ ($MH^+$) 309.1364, found 309.1361.

**3.1.13. 3-((1-(2-Chlorophenyl)-2-oxocyclohexyl)amino)-*N*,*N*-dimethylpropanamide hydrochloride (16)**. Similar reaction of **23** (0.30 g, 1.35 mmol) and 3-bromo-*N*,*N*-

dimethylpropanamide (0.48g, 2.70 mmol) with $K_2CO_3$/KI in MeCN at 100 °C for 48 h, followed by purification as above, gave a pale yellow oil (0.30 g, 70%) which was converted to **16** as above. $^1$HNMR ($CDCl_3$) δ 7.55-7.52 (dd, *J*= 7.84 Hz, 1.72 Hz, 1H), 7.38-7.35 (dd, *J*= 7.76 Hz, 1.48 Hz, 1H), 7.33-7.28 (td, *J*= 7.44 Hz, 1.52 Hz, 1H), 7.26-7.21 (td, *J*= 9.36 Hz, 1.76 Hz, 1H), 2.99 (s, 3H), 2.95 (s, 3H), 2.66-2.59 (m, 2H), 2.54-2.46 (m, 4H), 2.41-2.38 (m, 1H), 1.98-1.88 (m, 2H), 1.81-1.77 (m, 3H); $^{13}$C ($CDCl_3$) δ 207.50, 172.35, 139.57, 133.62, 131.28, 129.08, 128.58, 126.84, 69.44, 39.49, 38.82, 38.48, 37.34, 35.53, 33.98, 26.36, 21.78; MS *m/z* 323.20 ($MH^+$) Calculated for $C_{17}H_{24}ClN_2O_2$ ($MH^+$) 323.1521 found 323.1515

**3.1.14. 3-((1-(2-Chlorophenyl)-2-oxocyclohexyl)amino)-*N*-ethylpropanamide hydrochloride (17).** Similar reaction of **23** (0.35 g, 1.56 mmol) and 3-bromo-*N*-ethylpropanamide (0.56 g, 3.31 mmol) with $K_2CO_3$/KI in MeCN at 100 °C for 12 h, followed by purification as above, gave a pale yellow oil (0.36 g, 72%), which was converted to **17** as above. $^1$HNMR ($CDCl_3$) δ 7.55-7.52 (dd, *J*= 7.84 Hz, 1.72 Hz, 1H), 7.38-7.35 (dd, *J*= 7.76 Hz, 1.48 Hz, 1H), 7.33-7.28 (td, *J*= 7.44 Hz, 1.52 Hz, 1H), 7.26-7.21 (td, *J*= 9.36 Hz, 1.76 Hz, 1H), 7.14 (br, 1H), 3.23-3.13 (m, 2H), 2.95-2.89 (m, 1H), 2.65-2.59 (m, 1H), 2.55-2.45 (m, 2H), 2.38-2.25 (m, 4H), 1.86-1.62 (m, 4H), 1.07-1.03(t, *J*= 7.28 Hz, 3H); $^{13}$C ($CDCl_3$) δ 210.45, 172.46, 137.38, 134.16, 131.61, 129.56, 129.33, 127.11, 70.33, 39.81, 39.52, 38.26, 36.36, 34.17, 29.33, 22.26, 15.05; MS *m/z* 323.20 ($MH^+$) Calculated for $C_{17}H_{24}ClN_2O_2$ ($MH^+$) 323.1521, found 323.1509

**3.1.15. 3-((1-(2-Chlorophenyl)-2-oxocyclohexyl)amino)-N-isopropylpropanamide hydrochloride (18).** Similar reaction of **23** (0.20 g, 0.89 mmol) with 3-bromo-*N*-isopropylpropanamide (0.34 g, 1.80 mmol) with $K_2CO_3$/KI in MeCN at 110 °C for 48 h, followed by purification as above, gave a white solid (0.20 g, 67%) that was converted to **18** as above. $^1$HNMR ($CDCl_3$) δ 7.55-7.52 (dd, *J*= 7.84 Hz, 1.72 Hz, 1H), 7.38-7.35 (dd, *J*= 7.76 Hz, 1.48 Hz, 1H), 7.33-7.28 (td, *J*= 7.44 Hz, 1.52 Hz, 1H), 7.26-7.21 (td, *J*= 9.36 Hz, 1.76 Hz, 1H), 6.74 (br, 1H), 4.04-3.99 (m, 1H), 2.96-2.82 (m, 1H), 2.60-2.42 (m, 3H), 2.41-2.32 (m, 1H), 2.30-2.22 (m, 2H), 2.08-1.99 (m, 1H), 1.90-1.54 (m, 4H), 1.12-1.11 (d, *J*= 6.57 Hz, 3H), 1.06-1.05 (d, *J*= 6.60 Hz, 3H); $^{13}$C ($CDCl_3$) δ 210.12, 171.66, 137.69, 134.12, 131.62, 129.45, 129.22, 127.09, 70.26, 41.14, 39.84, 39.54, 38.53, 36.64, 28.98, 22.94, 22.85, 22.20; MS *m/z* 337.70 ($MH^+$) Calculated for $C_{18}H_{26}ClN_2O_2$ ($MH^+$) 337.1677 found 337.1676.

**3.1.16. 5-((1-(2-Chlorophenyl)-2-oxocyclohexyl)amino)-*N*-methylpentanamide hydrochloride (19) (Scheme 2).** Trimethylaluminium (4 mmol, 2M in hexanes) was slowly added to a suspension of methylamine hydrochloride (0.27g, 4.0 mmol) in toluene (4 mL) at -5 °C, and the resulting solution was warmed to room temperature and stirred for 2 h until no more gas evolved. A solution of ethyl 5-((1-(2-chlorophenyl)-2-oxocyclohexyl)amino)pentanoate (**24**) (0.34g, 0.98 mmol) in toluene (10 mL) was added to the above aluminium reagent and refluxed overnight. The reaction mixture was quenched with water and extracted with ethyl acetate and the organic layers were dried with magnesium sulphate. Column chromatography EtOAc/Hexanes (60-100%) afforded a pale yellow oil (0.24g, 72%), which was converted to **19** as above. $^1$HNMR ($CDCl_3$) δ 7.55-7.52 (dd, *J*= 7.84 Hz, 1.72 Hz, 1H), 7.38-7.35 (dd, *J*= 7.76 Hz, 1.48 Hz, 1H), 7.33-7.28 (td, *J*= 7.44 Hz, 1.52

Hz, 1H), 7.26-7.21 (td, *J*= 9.36 Hz, 1.76 Hz, 1H), 5.68 (br, 1H), 2.81-2.80 (d, *J*=3.77 Hz, 3H), 2.54-2.42 (m, 2H), 2.37-2.35 (m, 2H), 2.18-2.09 (m, 2H), 2.06-1.99 (m, 2H), 1.92-1.84 (m, 2H), 1.82-1.72 (m, 3H), 1.68-1.58 (m, 3H), 1.52-1.39 (m, 2H); $^{13}$C ($CDCl_3$) δ 209.54, 173.75, 138.33, 133.92, 132.12, 130.04, 129.50, 126.92, 70.22, 42.02, 39.74, 39.34, 36.36, 30.11, 28.30,26.48, 23.86, 22.08; MS *m/z* 337.20 ($MH^+$) Calculated for $C_{18}H_{25}ClN_2O_2$ ($MH^+$) 337.1677 found 337.1676.

**3.1.17. 5-((1-(2-Chlorophenyl)-2-oxocyclohexyl)amino)-*N*,*N*-dimethylpentanamide hydrochloride (20) (Scheme 2).** Trimethylaluminium (4 mmol, 2M in hexanes) was slowly added to a suspension of dimethylamine hydrochloride (0.33g, 4.1 mmol) in toluene (4 mL) at -5 $^o$C and the resulting solution was warmed to room temperature and stirred for 2 h until no more gas evolved. A solution of ethyl 5-((1-(2-chlorophenyl)-2-oxocyclohexyl)amino)pentanoate (**24**) (0.32g, 0.96 mmol) in toluene (10 mL) was added to the above formed aluminium reagent and refluxed overnight. The reaction mixture was worked up as for the preparation of **19** to give a pale yellow oil (0.22g, 71%), which was converted as above to **20**. $^1$HNMR ($CDCl_3$) δ 7.59-7.53 (dd, *J*= 7.77Hz, 1.72 Hz, 1H), 7.41-7.32 (m, 2H), 7.28-7.25 (td, *J*= 7.57 Hz, 1.76 Hz, 1H), 2.92-2.89 (d, *J*=14.0 Hz, 3H), 2.80-2.78 (d, *J*= 9.56 Hz, 3H), 2.40-2.28 (m, 2H), 2.27-2.18 (m, 3H), 1.94-1.82 (m, 4H), 1.74-1.68 (m, 2H), 1.66-1.38 (m, 5H); $^{13}$C ($CDCl_3$) δ 205.40, 171.87, 140.36, 132.42, 130.53, 129.36, 128.92, 126.88, 68.20, 42.14, 38.42, 38.10, 36.72, 34.77, 32.13, 29.67, 25.21, 22.44, 20.77; MS *m/z* 351.20 ($MH^+$) Calculated for $C_{19}H_{27}ClN_2O_2$ ($MH^+$) 351.18338 found 351.18272.

**3.1.18. 2-(2-Chlorophenyl)-2-((3-(piperidin-1-yl)propyl)amino)cyclohexan-1-one hydrochloride (21).** Similar reaction as in Scheme 1 of **23** (0.15 g, 0.67 mmol) and 1-(3-chloropropyl)piperidine hydrochloride (0.80 g, 4.04 mmol) with $K_2CO_3$/KI in MeCN at 110 $^o$C for 12 h, followed by purification as above, gave a pale yellow oil (0.12 g, 52%) which was converted to **21** as above. $^1$HNMR ($CDCl_3$) δ 7.71-7.65 (dd, *J*= 8.0 Hz, 1.56 Hz, 1H), 7.42-7.34 (m, 2H), 7.31-7.24 (td, *J*=7.24 Hz, 1.6 Hz, 1H), 3.15-3.05 (m, 2H), 3.00-2.85 (m, 3H), 2.51-2.45 (m, 3H), 2.45-2.35 (m, 2H), 2.10-1.90 (m, 7H), 1.80-1.70 (m, 4H), 1.72-1.65 (m, 3H); $^{13}$C ($CDCl_3$) δ 211.15, 136.71, 134.09, 131.29, 130.10, 129.50, 127.66, 70.96, 57.20, 53.76, 53.64, 53.42, 40.91, 40.86, 40.33, 29.64, 23.96, 23.38, 22.48, 22.11; MS *m/z* 349.20 ($MH^+$) Calculated for $C_{20}H_{29}ClN_2O$ ($MH^+$) 349.2041 found 349.2043

**3.1.19. 2-(2-Chlorophenyl)-2-((2-(ethylsulfonyl)ethyl)amino)cyclohexan-1-one hydrochloride (22)**.Similar reaction of **23** (0.44 g, 1.97 mmol) and 1-bromo-2-(ethylsulfonyl)ethane[14] (0.67 g, 3.30 mmol) with $K_2CO_3$/KI in MeCN at 120 $^o$C for 168 h, followed by purification as above, gave a pale yellow oil (0.46 g, 70 %) which was converted to **22** as above. $^1$HNMR ($CDCl_3$) δ 7.94-7.92 (m, 1H), 7.60-7.56 (m, 3H), 3.20-3.02 (m, 5H), 2.81-2.72 (m, 1H), 2.29-2.19 (m, 2H), 2.05-1.82 (m, 6H), 1.36-1.28 (t, *J*= 7.49 Hz, 3H); $^{13}$C ($CDCl_3$) δ 209.28, 135.22, 133.64, 133.20, 132.08, 131.99, 129.94, 69.06, 47.98, 47.42, 39.52, 37.94, 23.20, 22.39, 21.04, 6.77; MS *m/z* 344.10 ($MH^+$) Calculated for $C_{16}H_{23}ClNO_3S$ ($MH^+$) 344.1082 found 344.1078.

### 3.2. Biological activity

#### 3.2.1. General

All animal experiments were conducted at the Ruakura Research Centre, Hamilton, New Zealand, using experimental protocols reviewed and approved by the Ruakura Animal Ethics Committee (ethics ref 13420). Following acquisition of baseline physiologic parameters (heart rate, respiratory rate, PWR, and documentation of intact righting reflex (RR)) adult female Sprague-Dawley rats (age 119 to 168 days; weight 230 to 320g) were put under non-traumatic restraint and the marginal tail vein cannulated. Ketamine or an experimental compound at 10mg/ml was administered by automated syringe driver connected via a minibore extension tube adequately secured to the animal's tail. Infusions were commenced at a rate (weight-adjusted) to deliver 20 mg/kg/min initially (continued until loss of righting reflex (LORR) and subsequent pedal withdrawal reflex score PWR=1), then were reduced to a rate of 6.7 mg/kg/min. Infusion rate was then titrated in an up-and-down fashion to maintain dorsal recumbency and a PWR=1 to 10 minutes before cessation. Three rats were used in each study, with each group of rats also acting as their own ketamine control. The order of study drug administration was determined by prior odds/evens randomisation with a recovery interval of at least four hours afforded between experiments. PWR and RR were recorded at 1 minute intervals throughout. The times from cessation of infusion to return of righting reflex (RORR), and from cessation of infusion to the animals displaying independent locomotion (walk) were recorded.

**4.2.2. Pedal Withdrawal Reflex (PWR) scoring:** Nociceptive testing in animals was conducted via 1 second application of constant pressure (firm digital pressure) over the forepaw of the animal. Pedal withdrawal reflex testing is primarily used to assess analgesic effect, and responses are graded accordingly: 0, absent; 1, flicker; 2, moderate withdrawal; 3, fast withdrawal; 4, Fast withdrawal with cry/preceding apnoea (modified from ref 16).

**4.2.3. Loss of Righting Reflex (LORR):** This is primarily used to assess anaesthetic hypnotic effect. Righting reflex is judged absent when the rat fails to right from a position of dorsal recumbency to a position of sternal recumbency on three attempts performed in rapid succession. Dose to LORR is termed effective potency.

**4.2.4. Determination of NMDA inhibition:** Determinations of NMDA inhibition were carried out by Eurofins Panlabs Taiwan, Ltd. Pharmacology Laboratories 158 Li-Teh Road, Peitou Taipei, Taiwan 11259, in Wistar rat brain (minus cerebellum) preparations with MK-801 {[5*R*,10*S*]-[+]-5-methyl-10,11- dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine} as ligand, by radioimmunoassay, in 5 mM Tris-acetate buffer (pH 7.4) for 3 h at 25°C).

**Acknowledgements**

The authors thank the Biopharma Thematic Research Initiative, University of Auckland, and Kea Therapeutics Ltd, Auckland for funding.

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