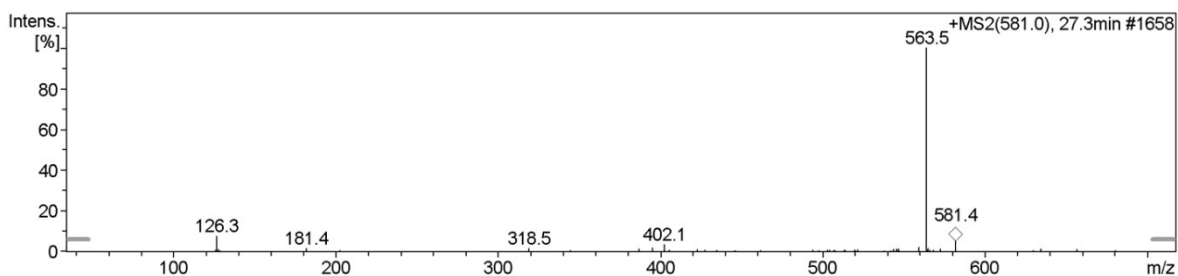


## Supplementary data

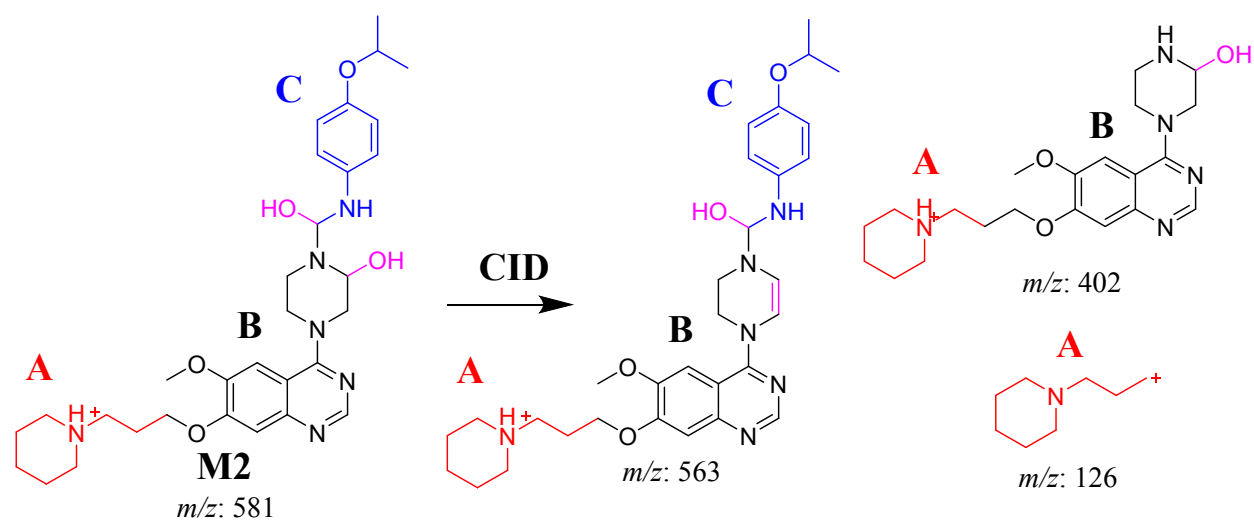
### 3.1. Identification of *in vitro* phase I TND metabolites.

#### 1.1. Identification of the M2 metabolite.

The M2 metabolite of TND was detected at ( $m/z$  581) in the MS scan mode at a  $t_R$  of 27.3 min. CID of MIPs at ( $m/z$  581) provided various product ions (Fig. S1). The product ion at ( $m/z$  126) indicated no metabolic reaction at part A. The product ion at ( $m/z$  402) indicated a hydroxylation metabolic reaction (increase of 16 in  $m/z$ ) at part B. The product ion at ( $m/z$  563) indicated dehydration (water loss) revealing hydroxylation at the piperazine moiety. The remaining metabolic reaction involves a reduction proposed at part C (reduction of the carbonyl moiety of the amide group) (Scheme S1).



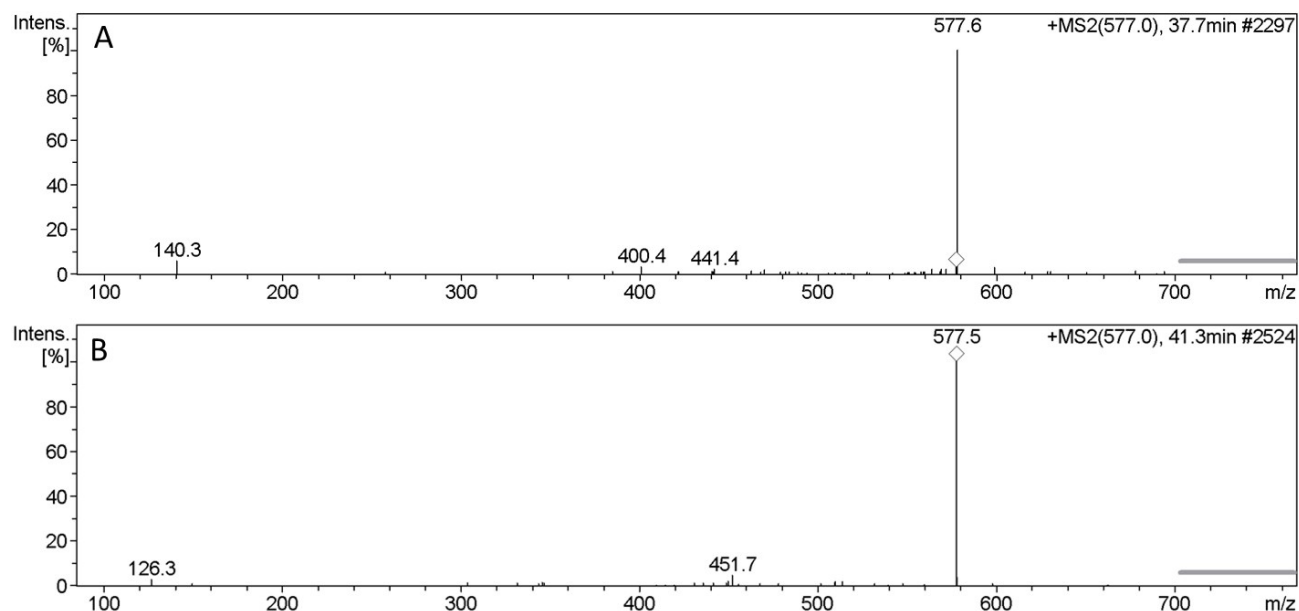
**Fig. S1.** MS<sup>2</sup> mass spectra of M2.



**Scheme S1:** Proposed CID of M2.

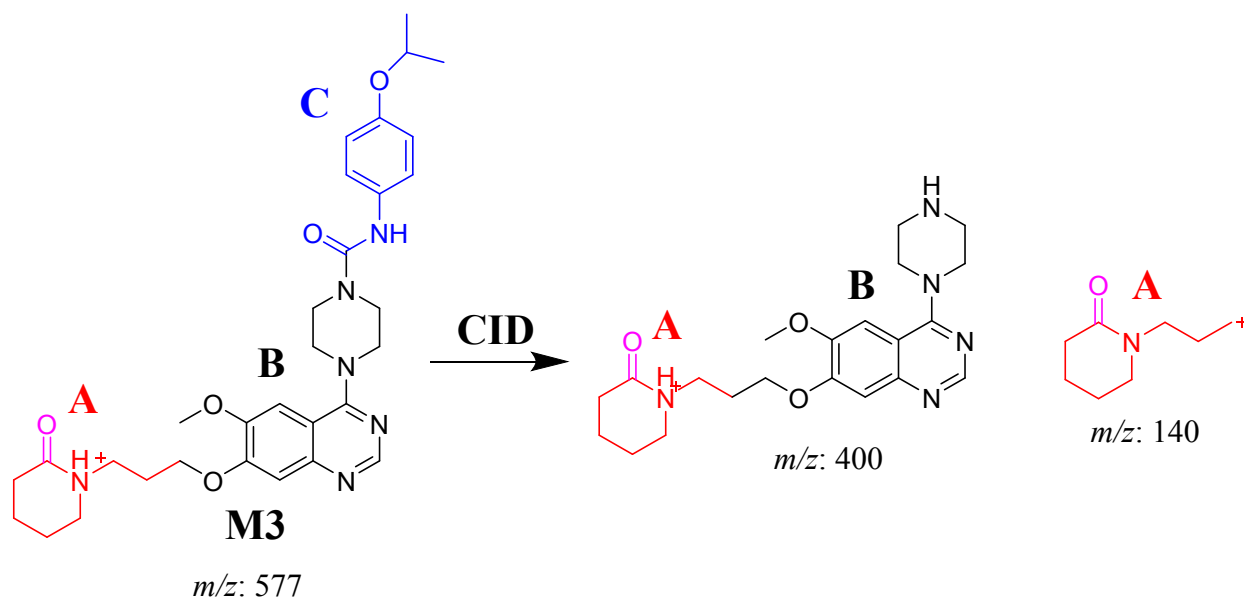
18 1.2. Identification of the M3 and M4 metabolites.

19 The M3 and M4 metabolites of TND were detected at ( $m/z$  577) in the MS scan mode at  
20  $t_R$  values of 37.7 and 41.3 min, respectively. The CID of MIPs at ( $m/z$  577) provided different  
21 product ions (Figs. S2A and S2B).



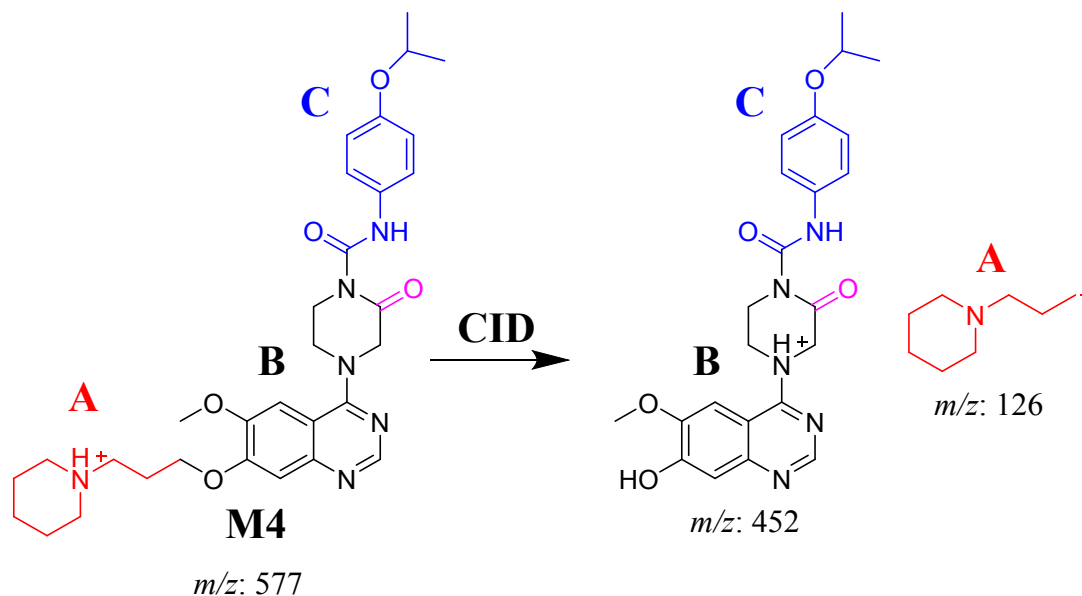
22  
23 **Fig. S2.** MS<sup>2</sup> mass spectra of M3 (A) and M4 at ( $m/z$  577) (B).

24 In the case of M3, in comparison with product ions of TND, product ions at ( $m/z$  140)  
25 and ( $m/z$  400) showed a 14  $m/z$  increase indicating an oxidation metabolic reaction at the  
26 piperidine ring (part A) (Scheme S2).



27  
28 **Scheme S2:** Proposed CID of M3.

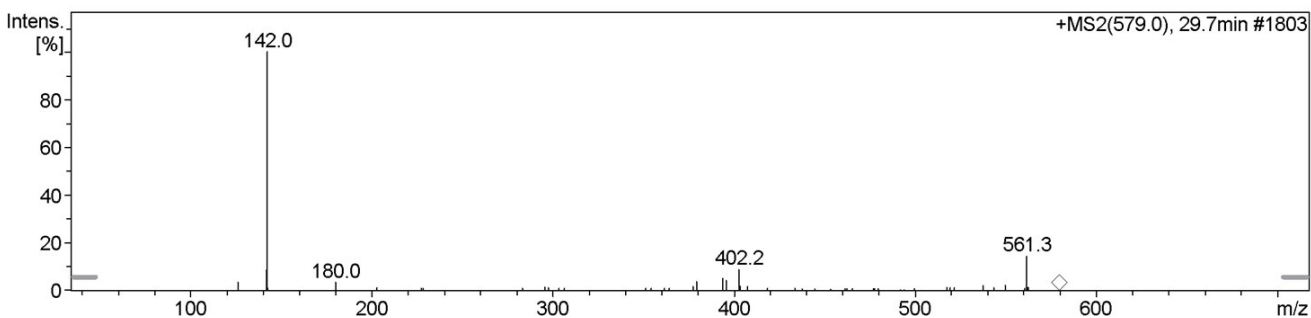
29 In the case of M4, by comparing to product ions of TND, product ion at ( $m/z$  126)  
30 indicated no metabolic reaction at part A that matched with the other product ion at ( $m/z$  452)  
31 (Scheme S3). The only possible remaining metabolic site is the oxidative metabolic reaction at  
32 the piperazine ring (part A)



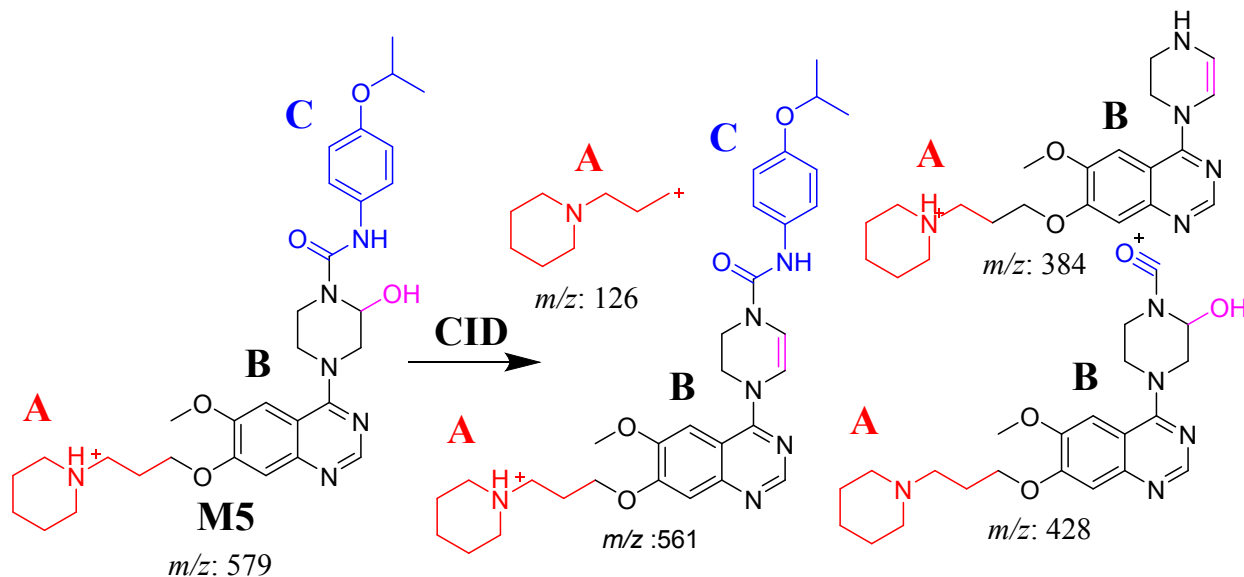
35

36 1.3. Identification of the M5 metabolite.

37 The M5 metabolite of TND was detected at ( $m/z$  579) in the MS scan mode at a  $t_R$  of 27.2 min.  
38 CID of MIPs at ( $m/z$  579) provided product ions at ( $m/z$  561), ( $m/z$  126), ( $m/z$  384), and ( $m/z$  428)  
39 (Fig. S3). The product ion at ( $m/z$  126) indicated no metabolic reaction at part A. The product ion  
40 at ( $m/z$  384) indicated water loss (decrease of 18  $m/z$ ) and a hydroxylation metabolic reaction at  
41 part B. The product ion at ( $m/z$  561) indicated water loss, revealing hydroxylation at the  
42 piperazine moiety. No metabolic reaction was expected at parts A or C (Scheme S4).



44 **Fig. S3.** MS<sup>2</sup> mass spectra of M5 at ( $m/z$  579).



## 48 2. Identification of TND *in vivo* phase I and phase II metabolites

### 49 2.1. Identification of the M8 metabolites of TND.

50 The M8 metabolite of TND was detected at ( $m/z$  579) in the MS scan mode at a  $t_R$  of 27.2 min.

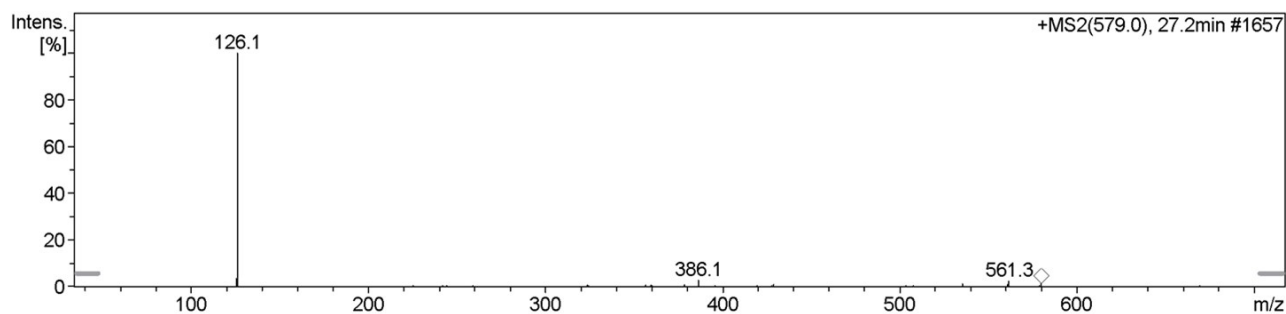
51 The CID of MIPs at ( $m/z$  579) generated product ions at ( $m/z$  386) and ( $m/z$  126) (Fig. S4). In

52 comparison to TND product ions, the product ion at ( $m/z$  126) indicated no metabolic reaction at

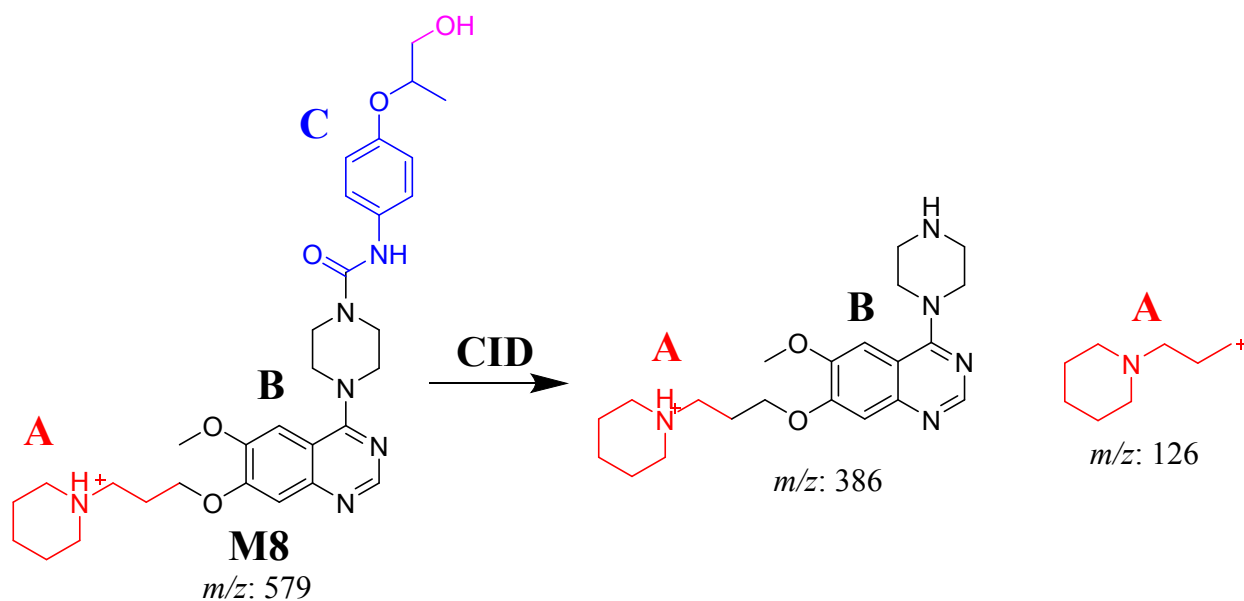
53 part A. The product ion at ( $m/z$  386) indicates no metabolic reaction at part B. The hydroxylation

54 metabolic reaction was predicted at part C, which is most likely at the isopropyl group and

55 matches with *in silico* predictions (Scheme S5).



59



62

63

64 2.2. Identification of the M9 metabolites of TND.

65 The M9 metabolite of TND was detected at ( $m/z$  591) in the MS scan mode at a  $t_R$  of 40 min.

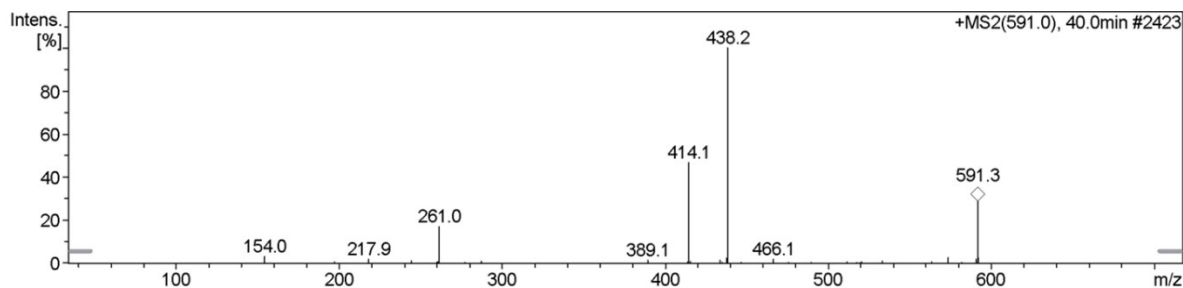
66 CID of the MIPs at ( $m/z$  591) generated product ions at ( $m/z$  438), ( $m/z$  414), and ( $m/z$  154) (Fig.

67 S5). In comparing to TND product ions, the product ion at ( $m/z$  438) indicated no metabolic

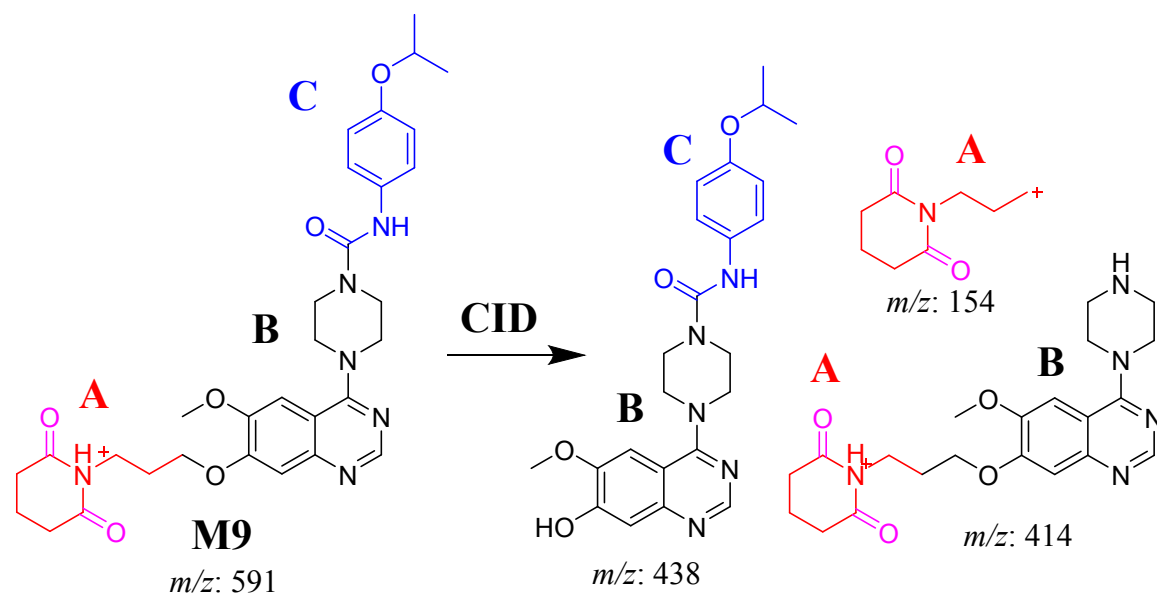
68 reaction at parts B or C. The product ion at ( $m/z$  154) indicated that the metabolic reaction

69 occurred at part A, which matched with the other product ion at ( $m/z$  414). Two  $\alpha$  oxidation

70 metabolic reactions were predicted at the piperidine ring of part A (Scheme S6).



71 **Fig. S5.** MS<sup>2</sup> mass spectrum of M9



72

73 **Scheme S6:** Proposed CID of M9.

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