Accepted Manuscript

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PII: S0223-5234(18)30497-5

DOI: 10.1016/j.ejmech.2018.06.007

Reference: EJMECH 10477

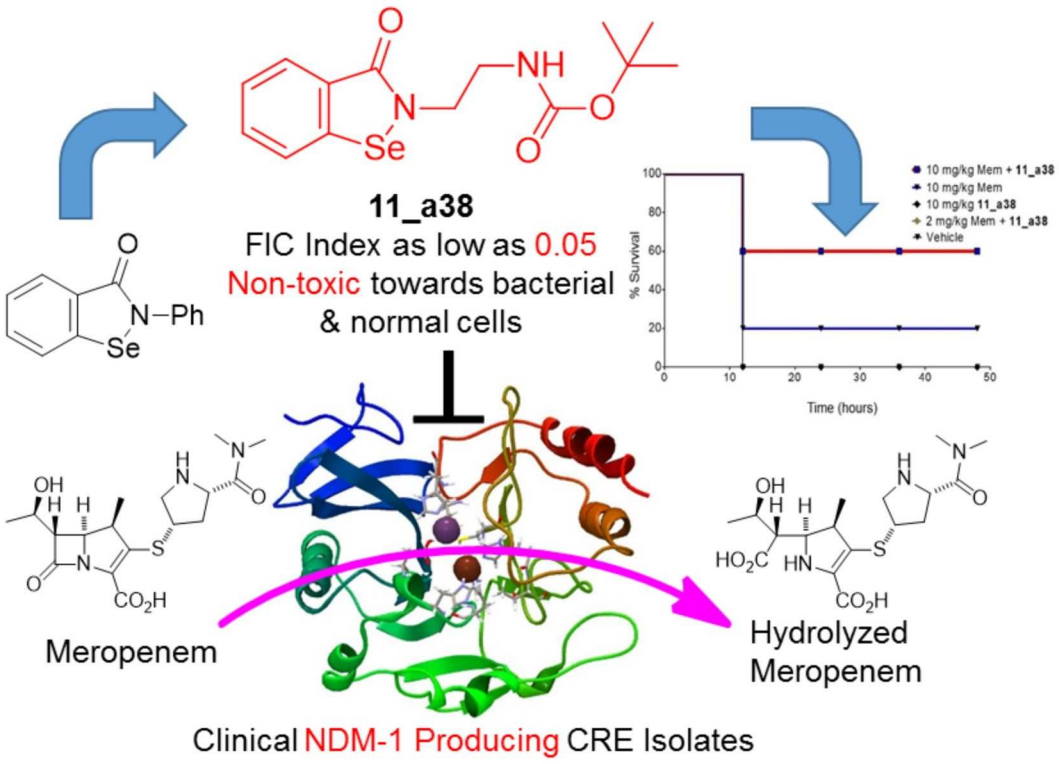
To appear in: *European Journal of Medicinal Chemistry*

Received Date: 7 February 2018

Revised Date: 11 April 2018

Accepted Date: 1 June 2018

Please cite this article as: W.B. Jin, C. Xu, Q. Cheng, X.L. Qi, W. Gao, Z. Zheng, E.W.C. Chan, Y.-C. Leung, T.H. Chan, K.-Y. Wong, S. Chen, K.-F. Chan, Investigation of synergistic antimicrobial effects of the drug combinations of meropenem and 1,2-benzisoselenazol-3(2*H*)-one derivatives on carbapenem-resistant *Enterobacteriaceae* producing NDM-1, *European Journal of Medicinal Chemistry* (2018), doi: 10.1016/j.ejmech.2018.06.007.



Investigation of Synergistic Antimicrobial Effects of the Drug Combinations of Meropenem and 1,2-Benzisoselenazol-3(2*H*)-one Derivatives on Carbapenem-Resistant *Enterobacteriaceae* Producing NDM-1

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ABSTRACT

The worldwide prevalence of NDM-1-producing bacteria has drastically undermined the clinical efficacy of the last line antibiotic of carbapenems, prompting a need to devise effective strategy to preserve their clinical value. Our previous studies have shown that ebselen can restore the efficacy of meropenem against a laboratory strain that produces NDM-1. Here we report the construction of a focused compound library of 1,2-benzisoselenazol-3(2*H*)-one derivatives which comprise a total of forty-six candidate compounds. The structure-activity relationship of these compounds and their potential to serve as an adjuvant to enhance the antimicrobial efficacy of meropenem against a collection of clinical NDM-1-producing carbapenem-resistant *Enterobacteriaceae* isolates was examined. Drug combination assays indicated that these derivatives exhibited synergistic antimicrobial activity when used along with meropenem, effectively restoring the activity of carbapenems against the resistant strains tested in a *Galleria mellonella* larvae *in vivo* infection model. The mode of inhibition of one compound, namely **11_a38**, which was depicted when tested on the purified NDM-1 enzyme, indicated that it could covalently bind to the enzyme and displaced one zinc ion from the active site. Overall, this study provides a novel 1,2-benzisoselenazol-3(2*H*)-one scaffold that exhibits strong synergistic antimicrobial activity with carbapenems, and low cytotoxicity. The prospect of application of such compounds as carbapenem adjuvants warrants further evaluation.

Keywords

Carbapenem-resistant *Enterobacteriaceae*, NDM-1 inhibitors, Ebselen derivatives, Carbapenem adjuvants, Synergistic activity, Drug combination

Introduction

Carbapenem-resistant *Enterobacteriaceae* (CRE) comprise a family of Gram-negative bacteria that exhibit antimicrobial resistance to multiple antibiotics, notably carbapenems which are the drugs of last resort for treating lethal infections. CRE are the major causes of community- and healthcare-associated bacterial infections, such as those in the urinary tract, bloodstream, surgical site, and intra-abdominal region. Because of their phenotypic resistance to various antibiotics, infections due to such “nightmare bacteria” are often difficult to treat, resulting in a significantly increased mortality. According to a recent report published by the Centers for Disease Control and Prevention (CDC), more than 9,000 hospitalized patients are infected by CRE annually and half of these patients who developed CRE-associated bloodstream infections died eventually.[1] Moreover, about 600 death cases are due to infection by the two most common types of CRE, carbapenem-resistant *Klebsiella* spp. and *E. coli*, annually. More importantly, recent surveillance reports on antimicrobial resistance also provided evidence which suggests that there is increasing prevalence of CRE strains in the European countries and China.[2-3] CRE infections have now been regarded as a serious threat to public health. This situation has been be further deteriorated with the recent emergence of MCR-1 positive, colistin-resistant CRE, which do not respond to all known antibiotics.[4-6] A list of antibiotic-resistant "priority pathogens" released by the World Health Organization (WHO) early this year indicates that CRE are among the most critical group of bacteria which cause infections that require new antibiotics for treatment [7].

One of the principal resistance mechanisms of carbapenems in CRE involves the production of carbapenemases, which are carbapenem-hydrolyzing β-lactamases that possess versatile hydrolytic capacities to break down nearly all β-lactam antibiotics and render them ineffective.[8]

These broad-spectrum carbapenemases belong to members of the class A, B, and D β-lactamases, among which the most efficient carbapenemases are class B metallo-β-lactamases (MBLs).[9] MBLs employ a central zinc ion as essential cofactor to catalyze hydrolysis of the β-lactam ring. The hydrolytic process of β-lactam antibiotics does not involve any covalent enzyme-β-lactam intermediate but rather a nucleophilic attack on the water/hydroxyl ion which is stabilized by the divalent zinc ion in the active site. MBLs can be further divided into three subclasses (B1, B2 and B3) according to their amino acid sequence. As illustrated in **Figure 1**, for example, New Delhi metallo-β-lactamase-1 (NDM-1) of subclass B1 MBLs contains a dinuclear zinc centre and a water molecule in the active site, in which both zinc ions are coordinated with the 3H site (His116, His118, His196) and the DCH site (Asp120, Cys221, His263), and the water molecule is located in between both zinc ions.[10] Clinically, subclass B1 MBLs are the most significant and prevalent carbapenemases, in particular, NDM-1. The rapid worldwide dissemination of NDM-1-producing "superbugs" further emphasizes the significant role of this type of carbapenemases in conferring antimicrobial resistance, and a pressing need for development of effective NDM-1 inhibitors for clinical applications.[11]

Despite the discovery of a NDM-1-producing *K. pneumoniae* isolate in year 2009[12] , huge success has been made in combating organisms that produce β-lactamases through clinical usage of β-lactam antibiotic/β-lactamase inhibitor combinations.[13] In particular, development of small molecules targeting NDM-1 have been actively pursued in the past decade.[14] Numerous reports on construction of NDM-1 inhibitors such as cyclic boronate **1**,[15] dicarboxylate heterocycle **2**,[16] thiol-containing carboxylic acids **3**, **5-6**,[10, 17-18] metal chelator **4**,[19-20] natural product **7**[21] and covalent inhibitors like cefaclor[22] and chromone-3-carboxaldehyde,[23] have appeared in the

literature (**Figure 1**). Despite the ongoing effort, clinically useful inhibitors of MBLs, in particular NDM-1, are still not available. Development of clinically useful NDM-1 inhibitors has been deemed technically challenging, with compound toxicity being a great concern.

We have previously identified a promising small molecule, covalent NDM-1 inhibitor, namely ebselen (Eb) (**Figure 1**), which possesses an active scaffold of 1,2-benzisoselenazol-3(2*H*)-one, through a cell-based screening assay against a laboratory strain of *E. coli* BL21 (NDM-1) that harbors an isopropyl-β-D-thiogalactoside (IPTG) inducible full-length plasmid pET28b-bla_{NDM-1}.[24] Eb itself does not exhibit antibacterial activity towards the test strain. However, it exhibits potent synergistic activity when used in combination with meropenem (Mem) and re-sensitizes the resistant strain to a level equivalent that of the parental, non-NDM-1-producing *E. coli* BL21 strain, suggesting that it has great potential to be further developed into a new class of carbapenem adjuvant. Despite its potent synergistic activity against the laboratory strain of *E. coli* BL21 (NDM-1), further investigation of Eb revealed that its potency of inhibition towards clinically isolated NDM-1 producing CRE strains was not as potent as expected. As shown in **Table 2**, the minimum inhibition concentrations (MIC) screening of Mem in combination with Eb against a panel of twenty-three clinical NDM-1 producing CRE isolates evidenced that the MIC values of Mem only ranged from 16 μg/mL to 128 μg/mL, suggesting that the inhibitory potency was only mild to moderate. The synergistic antimicrobial activity of Eb when used in combination with meropenem was exhibited only in six clinical CRE isolates, with fractional inhibitory concentration (FIC) indices being less than 0.5 (**Table 2**, Entry 4-6, 9-10 and 13). Moreover, attempts to dissolve Eb in various aqueous formulations for *in vivo* efficacy assay was found to be impractical. We reasoned that this might be due to the inferior ability of *E. coli* BL21

(NDM-1) in mimicking clinical CRE strain physiologically, and the intrinsically sub-optimal drug-like properties of Eb.

In the present study, using Eb as a molecular template, we have constructed a focused compound library with forty-four candidates of 1,2-benzisoselenazol-3(2*H*)-one derivatives and two structurally related compounds, with the aims of exploring the structure-activity relationship (SAR) of these potential carbapenem adjuvants and improving their physicochemical properties. Another *E. coli* strain, Tg1 (NDM-1), is also generated to replace *E. coli* BL21 (NDM-1) for preliminary cell-based screening assay. By evaluating the FIC indices of these derivatives when tested in combination with Mem, the identified promising hits were selected for MIC screening against clinical NDM-1 producing CRE isolates and for standard enzymatic assays to confirm their inhibitory effect on the purified NDM-1 enzyme. The most effective derivative was further tested to assess their *in vivo* efficacy and cytotoxicity on eukaryotic cells. By using electrospray ionization mass spectrometry (ESI-MS) analysis, its mechanism of NDM-1 inhibition was also investigated in details.

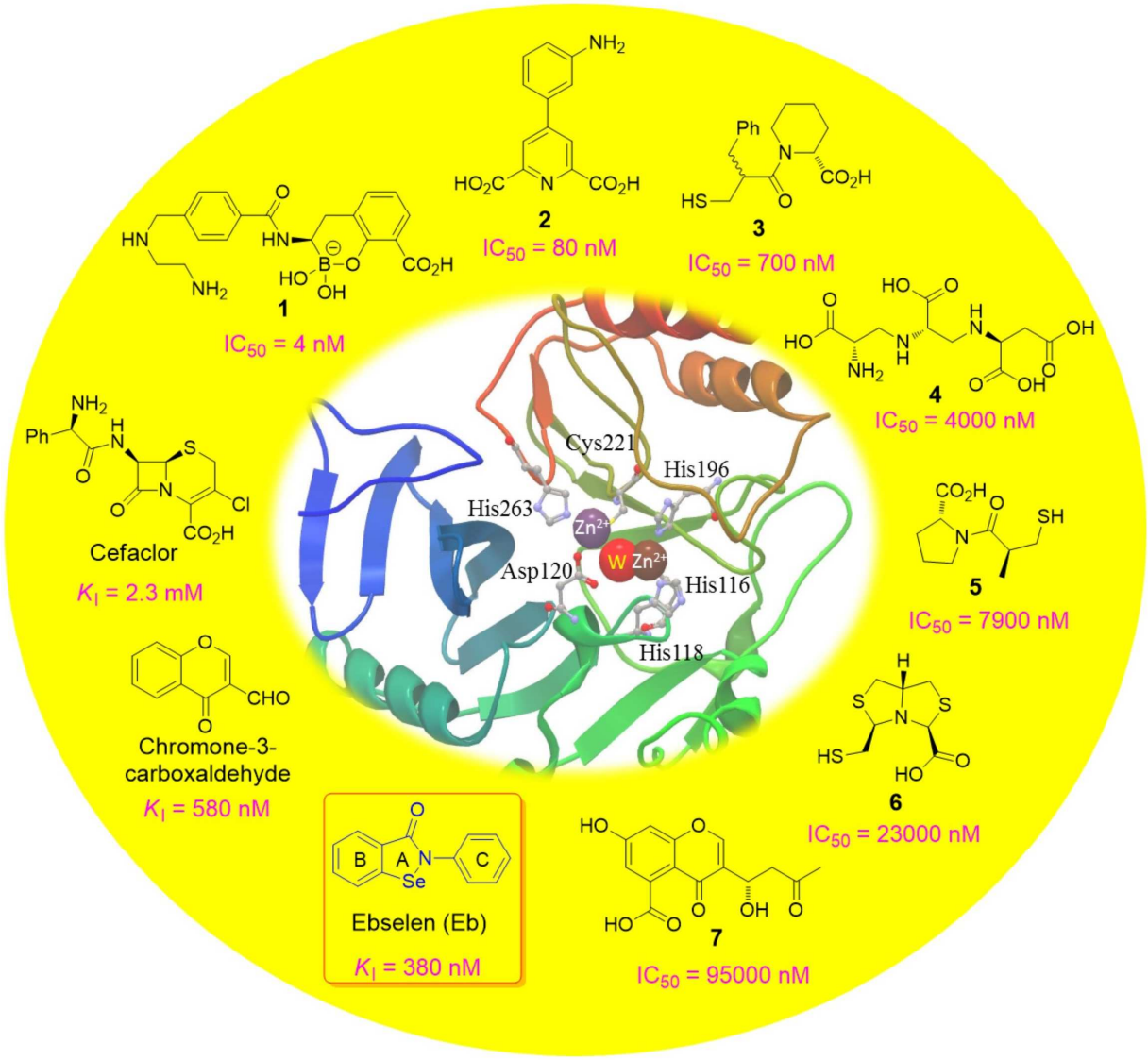


Figure 1. Crystal structure of NDM-1 (PDB ID: 3Q6X) showing the active site of dinuclear zinc centre and water (W), along with the numbered functionally important amino acids residues; the chemical structures of various NDM-1 inhibitors and their reported values of IC_{50} or K_I were highlighted in red; Eb was labelled with the A, B and C rings.

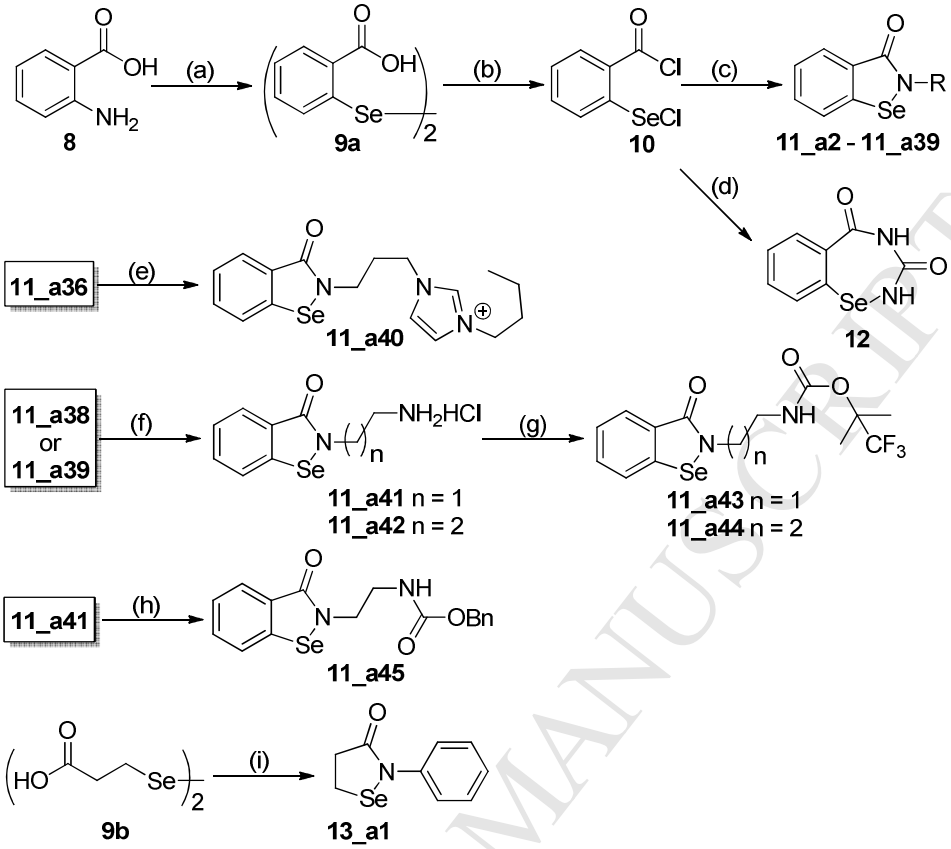
Results and Discussion

1. Chemical synthesis

The synthetic routes of the target compound library of 1,2-benzisoselenazol-3(2*H*)-one derivatives **11** are straightforward and concise, and depicted in **Scheme 1**.[25] Starting from commercially available anthranilic acid (**8**), diazotization with sodium nitrite in hydrochloric acid followed by treatment with a basified solution of disodium diselenide (Na_2Se_2) under inert atmosphere afforded the 2,2'-diselanediyldibenzoic acid (**9a**) in good yield. It is worthy to note that the water used to prepare Na_2Se_2 solution should first be degassed to remove oxygen. Further conversion of acid **9a** to 2-(chlorocarbonyl)phenyl hypochloroselenoite (**10**) via treatment with thionyl chloride at refluxing temperature in the presence of a catalytic amount of dimethylformamide (DMF) was achieved in high yield. It is worthy to mention that the acid chloride **10** is unstable and should be kept under the nitrogen atmosphere in a freezer for prolonged storage. The synthesis of the final 1,2-benzisoselenazol-3(2*H*)-one derivatives **11** were successfully accomplished via treatment of acid chloride **10** with various aryl or alkyl substituted primary amines **a1**-**a14**, **a17**-**27**, **a29**-**a36**, **a38**-**a39**, amide **a15**, and sulfonamide **a16** (**Chart 1**) respectively in the presence of triethylamine with dichloromethane (DCM) as solvent, producing moderate to good yield. Similarly, treatment of acid chloride **10** with D-Ala-D-Ala (**a28**) or glycine (**a37**) in acetonitrile at room temperature furnished **11** with a carboxyl acid moiety. Moreover, three derivatives of **11** were selected for further functionalization, such as alkylation of **11_a36** with 1-butyl iodide in DMF to furnish **11** with an imidazolium moiety, and acidic deprotection of Boc group of **11_a38** and **11_a39** to yield **11** with an amine group. These amine derivatives were in turn converted to the desired carbamates derivatives **11_a43**-**11_a45** via standard carbamoylation procedure with 3-methyl-1-(((1,1,1-trifluoro-2-methylpropan-2-

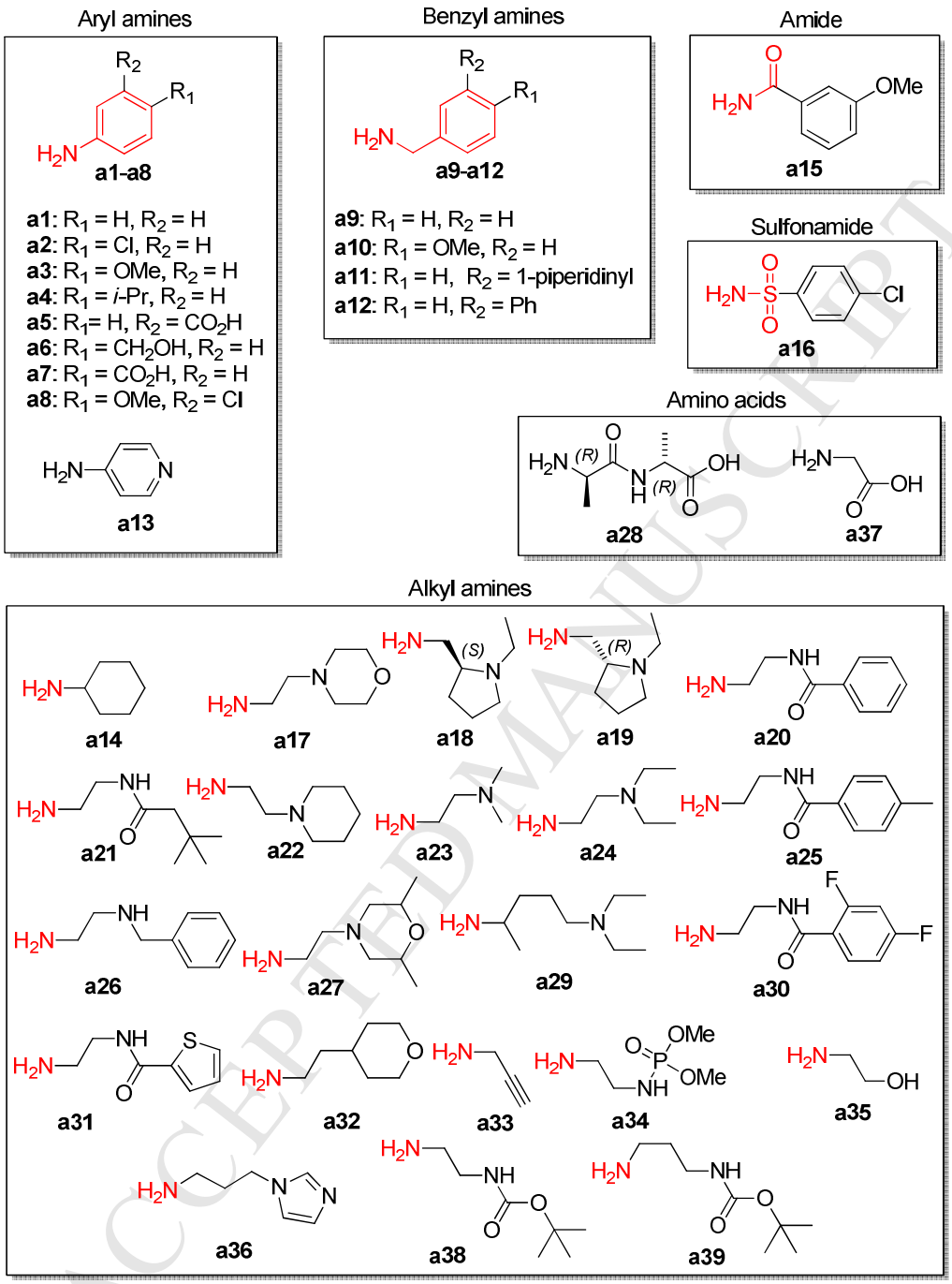
yl)oxy)carbonyl)-1*H*-imidazol-3-ium iodide or benzyl chloroformate. Furthermore, two structurally related compounds benzo[*f*][1,2,4]-selenadiazepine-3,5(2*H*,4*H*)-dione (**12**) and 2-phenyl-1,2-selenazolidin-3-one (**13_a1**) were also synthesized for testing. Treatment of acid chloride **10** with urea in DCM afforded the compound **12** with a characteristic seven-members 1,2,4-selenadiazepane ring in moderate yield.[26] In the case of compound **13-a1**, starting from commercially available 3,3'-diselanediyldipropanoic acid (**9b**), *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide (EDCI) catalyzed condensation of acid **9b** with aniline (**a1**), followed by *tert*-butyl hydrogen peroxide assisted cyclization, smoothly generated the desired 1,2-selenazolin-3-one **13_a1** in low yield.

Scheme 1. Chemical synthesis of 1,2-benzisoselenazol-3(2*H*)-one derivatives and structurally related compounds[a].



[a] (a) (1) $NaNO_2$, conc. HCl (aq), 0 °C, 30 min; (2) Na_2Se_2, H_2O, 0 °C to 60 °C., 14 h; (b) $SOCl_2$, DMF (cat.), 0 °C to reflux, 3 h; (c) For compound **11_a2-11_a27**, **11_a29-11_a36**, **11_a38-11_a39**, amine **a2-a27**, **a29-a36**, **a38-a39,** NEt_3, DCM, rt, 14 h; For compound **11_a28** and **11_a37**, D-Ala-D-Ala (**a28**) and glycine (**a37**), ACN, rt, 48 h; (d) urea, DCM, acetonitrile, 0 °C to rt, 24 h; (e) 1-butyl iodide, DMF, rt, 14 h; (f) conc. HCl, MeOH, DCM, 0 °C to rt, 14 h; (g) 3-methyl-1-(((1,1,1-trifluoro-2-methylpropan-2-yl)oxy)carbonyl)-1*H*-imidazol-3-ium iodide, NEt_3, $CHCl_3$, 14 h; (h) benzyl chloroformate, NEt_3, DCM, 0 °C to rt, 14 h; (i) (1) amine **a1**, EDCI, DCM, 0 °C to rt, 14 h; (2) *t*-BuOOH, MeOH, rt, 24 h.

Chart 1. Chemical structures of aryl amines, benzyl amines, amide, sulfonamide, amino acids used in this study.



1

2

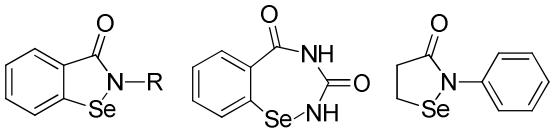
3 *2. Antimicrobial activities against E. coli Tg1 (NDM-1)*

4 To generate a Mem resistant strain with a clean background for preliminary screening of

5 compounds, a parental *E. coli* Tg1 strain was transformed into a resistant strain of *E. coli* Tg1

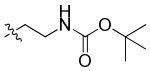
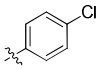
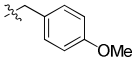
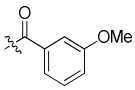
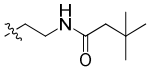
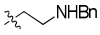
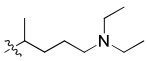
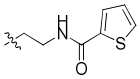
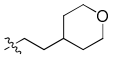
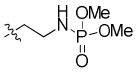
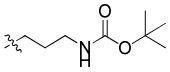
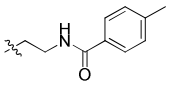
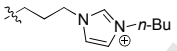
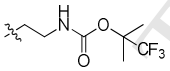
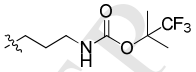
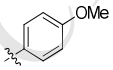
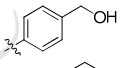
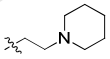
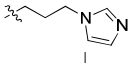
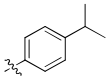
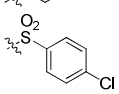
(NDM-1) by introducing a full-length bla_{NDM-1} β-lactamase gene isolated from a clinical *K. pneumoniae* isolate. *E. coli* Tg1 (NDM-1) is believed to better mimic clinical CRE strain than *E. coli* BL21 (NDM-1). In line with the Clinical and Laboratory Standards Institute (CLSI) approved guidelines,[27] antimicrobial tests were performed accordingly and the reproducibility was within one 2-fold dilution. The MIC of Mem against this screening strain was found to be 64 µg/mL (**Table 1**, entry 1), which was 128-fold higher than the parental *E. coli* Tg1 (MIC of Mem = 0.5 µg/mL), demonstrating that this strain was capable of producing the NDM-1 enzyme. The antimicrobial activities of all compounds were evaluated by measuring the MICs against this screening strain, and the results are presented in **Table 1**. In general, most of these compounds exhibited relatively weak or even no antibacterial activity, with MICs ≥64 µg/mL (Entry 3-42), suggesting that these compounds were well-tolerated, relatively less toxic and exhibiting little off-target side effects. However, six compounds (Entry 43-48) were found to exhibit mild antibacterial activity, with MIC values ranging from 4 µg/mL to 32 µg/mL, indicating that these compounds are likely to undergo non-specific interaction with protein targets other than NDM-1, and are not worthy to pursue further. Structurally, these compounds possess polar functional groups, such as alcohol, amine and carboxylic acid; an exception is compound **11_a9**, which has a benzyl group.

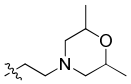
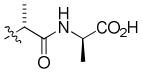
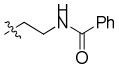
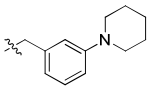
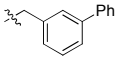
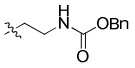
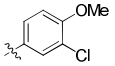
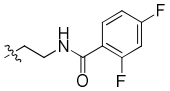
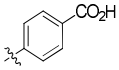
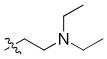
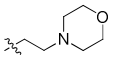
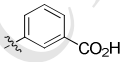
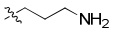
Table 1. MIC screening of compounds **11-13**, Eb and their combination with Mem against *E. coli* Tg1 (NDM-1), calculated cLogP, FIC indices and synergistic efficiency of compounds [a]



11_a2 - 11_a45 **12** **13_a1**

Entry	Cpd	R	cLogP	MIC (µg/mL)	FIC	Synergistic

				Cpd	Cpd + Mem	Index	Efficiency
1	Mem	N.A.	N.A.	64	N.A.	N.A.	N.A.
2	Eb		3.71	64	16	0.500	0.043
3	**11_a38**		3.04	≥128	2	0.047	0.153
4	**11_a2**		4.42	≥128	4	0.094	0.139
5	**11_a10**		3.65	≥128	8	0.188	0.088
6	**11_a15**		3.31	≥128	8	0.188	0.084
7	**11_a21**		2.95	≥128	8	0.188	0.084
8	**11_a26**		3.45	≥128	8	0.188	0.084
9	**11_a29**		3.71	≥128	8	0.188	0.084
10	**11_a31**		2.66	≥128	8	0.188	0.084
11	**11_a32**		2.74	≥128	8	0.188	0.084
12	**11_a34**		0.77	≥128	8	0.188	0.084
13	**11_a39**		3.33	≥128	8	0.188	0.080
14	**11_a25**		3.32	≥128	8	0.188	0.076
15	**11_a40**		-0.24	≥128	8	0.188	0.076
16	**11_a43**		4.68	≥128	8	0.188	0.073
17	**11_a44**		4.97	≥128	8	0.188	0.070
18	**11_a23**		2.11	≥128	16	0.375	0.065
19	**11_a3**		3.62	≥128	16	0.375	0.054
20	**11_a6**		2.67	≥128	16	0.375	0.054
21	**11_a22**		3.16	≥128	16	0.375	0.054
22	**11_a36**		2.01	≥128	16	0.375	0.054
23	**11_a4**		5.13	≥128	16	0.375	0.052
24	**11_a16**		3.28	≥128	16	0.375	0.049

25	**11_a27**		2.89	≥128	16	0.375	0.049
26	**11_a28**		1.71	≥128	16	0.375	0.049
27	**11_a20**		2.82	≥128	16	0.375	0.047
28	**11_a11**		4.57	≥128	16	0.375	0.043
29	**11_a12**		5.62	≥128	16	0.375	0.043
30	**11_a45**		3.52	≥128	16	0.375	0.043
31	**13_a1**	N.A.	2.52	≥128	32	0.750	0.024
32	**11_a13**		2.21	≥128	32	0.750	0.018
33	**11_a8**		4.25	≥128	32	0.750	0.015
34	**11_a30**		2.97	≥128	32	0.750	0.013
35	**11_a7**		3.45	64	4	0.125	0.109
36	**11_a33**		2.06	64	8	0.250	0.107
37	**12**	N.A.	0.84	64	8	0.250	0.107
38	**11_a14**		3.99	64	8	0.250	0.087
39	**11_a24**		3.17	64	16	0.500	0.041
40	**11_a17**		2.60	64	16	0.500	0.039
41	**11_a19**		3.12	64	16	0.500	0.039
42	**11_a18**		3.12	64	16	0.500	0.039
43	**11_a9**		3.73	32	8	0.375	0.058
44	**11_a5**		3.45	32	8	0.375	0.052
45	**11_a35**		1.16	32	16	0.750	0.022
46	**11_a37**		1.39	8	2	0.281	0.091
47	**11_a42**		1.55	8	2	0.281	0.091
48	**11_a41**		1.24	4	4	1.063	-0.005

[a]Compound cLogP were calculated using ChemDraw Ultra (Version 12.0). The synergistic effect is determined by the FIC index, which is calculated as FIC (cpd) + FIC (Mem), where FIC (cpd) is the (MIC of cpd in combination with Mem)/(MIC of cpd alone) while FIC (Mem) is (MIC of cpd in combination with Mem)/(MIC of Mem alone). The drug combination is considered synergistic if the FIC Index is ≤ 0.5. Synergistic efficiency is calculated by measuring

–ln(FIC index)/non-hydrogen atom. N.A.: Not Applicable. All experiments were performed in at least triplicates and inhibition of bacterial growth was assessed by naked eye upon incubation overnight.

3. Combination studies with Mem against E. coli Tg1 (NDM-1) and clinical NDM-1 producing CRE isolates

Next, to assess the synergistic activities of the test compounds when used in combination with Mem, MICs of Mem were evaluated by mixing Mem with the compounds at a mass ratio of 1:1. The FIC indices, which quantified the degree of interaction between antibiotic and adjuvant, were calculated as previously described,[28] with FIC indices ≤0.5 depicting synergistic interaction. To better differentiate the compounds which have exactly the same FIC index values, here we proposed, for the first time, a simple idea of synergistic efficiency, which is a similar concept to the ligand efficiency as previously proposed.[29] The synergistic efficiency is proposed to be calculated by using the equation as shown below,

$$\text{Synergistic efficiency} = \frac{-\ln(FIC\ index\ of\ a\ molecule)}{number\ of\ heavy\ atom\ in\ the\ molecule}$$

, where the heavy atom means non-hydrogen atom in a molecule. In fact, synergistic efficiency is a normalized FIC index to enable comparisons of synergistic activities between different molecular scaffolds but with the same FIC index. The larger the value of the synergistic efficiency, the stronger the synergistic interaction will be. It provides a useful parameter for medicinal chemists to choose a lead compound for further optimization. The results of Mem MICs when tested in combination with the compounds, cLogP, calculated FIC indices and synergistic efficiencies of all compounds are summarized in **Table 1**, in which compounds were prioritized according to the FIC indices and synergistic efficiency respectively. The parental compound Eb was used as a positive control for comparison purpose. The MIC of Mem in

combination with Eb was 16 μg/mL with a calculated FIC index of 0.50 and synergistic efficiency of 0.043 (**Table 1**, entry 2), exhibiting only moderate synergistic activity. Generally, most of the compounds were found to exhibit stronger synergistic activity than Eb, with FIC indices ranging from 0.047 to 0.375. These compounds were divided into two series to investigate their SARs: (1) 1,2-benzisoselenazol-3(2*H*)-one derivatives with a 2-aryl or benzyl side chain and (2) 1,2-benzisoselenazol-3(2*H*)-one derivatives with a flexible 2-alkyl side chain.

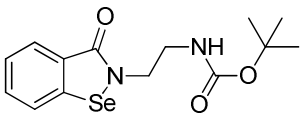
For series 1, installation of various functional groups on the phenyl ring C of Eb, such as 4-Cl, 4-MeO, 4-CH_2OH, 3-CO_2H, 4-CO_2H, and 4-^{i}Pr improved the synergistic activities dramatically with FIC indices ranging from 0.094 to 0.375 (Entry 4, 19, 20, 23, 35, 44). Furthermore, replacing the phenyl ring C of Eb with different benzyl groups also helped improve the synergistic activities (Entry 5, 28, 29, 43). It is worthy to mention that replacing the freely rotatable benzylic carbon with a carbonyl or sulfonyl group of high rigidity maintained the synergistic activities (Entry 6, 24). Compound **12** with an unusual seven-members 1,2,4-selenadiazepane ring also exhibited synergy (Entry 37). On the other hand, removal of the phenyl ring B or replacing the phenyl ring C of Eb with pyridine ring or 3-Cl-4-MeO di-substituted phenyl ring resulted in no synergistic activities (Entry 31-33). Among these derivatives, it should be mentioned that compound **11_a2** with a 4-Cl phenyl group demonstrated the most promising synergy, with a FIC index of 0.094 (Entry 4). However, its relatively high cLogP value of 4.42 revealed that it has lower hydrophilicity and poorer aqueous solubility than Eb, which may impede *in vivo* efficacy study.

For series 2, replacing the 2-phenyl ring of Eb with various flexible alkyl side chains generated many compounds of promising synergistic activities, with FIC indices ranging from 0.047 to 0.500 (Entry 3, 7-18, 21-22, 25-27, 30, 36, 38-42). Among these compounds, compound **11_a38** exhibited the most promising synergistic activity, with a FIC index of 0.047 (Entry 3), which is about 10-fold that of Eb. At a concentration of 2 μg/mL, it could re-sensitize Mem resistant screening strain back to the susceptible level. The bulky Boc group of **11_a38** was essential in conferring synergistic activity as its replacement with other functional groups, such as 3,3-dimethylbutanone (Entry 7), benzyl group (Entry 8), 2-thiophenylmethone (Entry 10), dimethylphosphonate (Entry 12), trifluoro-Boc (Entry 16), benzyl carbamate (Entry 30) and substituted phenylmethanones (Entry 14, 27, 34), resulted in weaker synergistic activities. Installation of heterocyclic rings at the terminal position, like 4-tetrahydropyran (Entry 11), *n*-butyl imidazolium (Entry 15), 1-piperidine (Entry 21), 1-imidazole (Entry 12) and 4-morpholine (Entry 25, 40), also caused weaker synergy. The CH_2CH_2 group of **11_a38** seems to be crucial for strong synergistic activity as increasing its length to $CH_2CH_2CH_2$ resulted in weaker synergy (Entry 13).

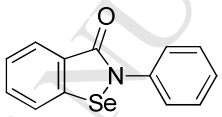
On the basis of the favorable FIC index of compound **11_a38** and MIC of Mem, we tested whether the observed synergy in the screening strain could also be reproduced in our in-house collection of clinically isolated NDM-1-producing strains, including four *E. coli*, two *K. oxytoca*, four *C. freundii*, nine *E. cloacae*, two *K. pneumoniae* and two *M. morganii* strains. These CRE strains were all NDM-1 positive and highly Mem resistant, with half of the strains exhibiting Mem MICs ≥128 μg/mL (**Table 2**). Apart from producing NDM-1, several of these strains also produced other β-lactamases such as VIM-1, IMP-4, KPC-2, CTX-M-3, CTX-M-14, CTX-M-15,

TEM-1 and SHV-12. As illustrated in **Table 2**, compound **11_a38** demonstrated no antibacterial activity itself (MICs ≥128 μg/mL) but strong synergy with Mem across this panel of clinical isolates, except for KP04 and EL18, with FIC indices ranging from 0.09 to 0.25 (**Table 2**, entry 1, 17). Compared with the parental compound Eb, compound **11_a38** exhibited much stronger synergy as well as the optimal cLogP value of 3.04 for the *in vivo* efficacy assay.

Table 2. MIC screening of compound **11_a38** and Eb in combination with Mem against clinically isolated, NDM-1-producing CRE strains[a]



11_a38



Eb

Entry	CRE Strains	Additional β-lactamase determinants	MIC (μg/mL)			FIC index	MIC (μg/mL)		FIC index
			Mem	**11_a38**	**11_a38** + Mem		Eb	Eb + Mem	
1	KP04	b5, b1, b7, b8	≥128	≥128	32	0.50	≥128	64	1.00
2	KP14	b5, b3, b8	≥128	≥128	8	0.13	≥128	≥128	2.00
3	EC06	b4, b5, b8	≥128	≥128	8	0.13	≥128	64	1.00
4	EC33	b6	64	≥128	8	0.19	≥128	16	0.38
5	EC34	b6, b7, b8	64	≥128	4	0.09	≥128	16	0.38
6	EC45	none	≥128	≥128	4	0.06	≥128	16	0.25
7	KO03	b8	≥128	≥128	8	0.13	≥128	32	0.50
8	KO16	b8	≥128	≥128	8	0.13	≥128	32	0.50
9	CF05	b8	≥128	≥128	8	0.13	≥128	16	0.25
10	CF17	b8	64	≥128	4	0.09	≥128	16	0.38
11	CF35	b4, b3, b8	≥128	≥128	8	0.13	≥128	32	0.50
12	CF43	none	≥128	≥128	16	0.25	≥128	32	0.50
13	EL07	b5, b7, b8	64	≥128	8	0.19	≥128	16	0.38
14	EL08	b5, b7, b8	32	≥128	4	0.16	≥128	32	1.25
15	EL09	b4, b5, b7, b8	≥128	≥128	8	0.13	≥128	≥128	2.00
16	EL10	b5, b7, b8	64	≥128	8	0.19	≥128	64	1.50
17	EL18	b2, b7	32	≥128	16	0.63	≥128	64	2.50
18	EL19	b5, b3	≥128	≥128	16	0.25	≥128	≥128	2.00

19	EL22	b5, b7, b8	≥128	≥128	16	0.25	≥128	≥128	2.00
20	EL27	b4, b5, b7, b8	32	≥128	4	0.16	≥128	64	2.50
21	EL28	b7, b8	32	≥128	4	0.16	≥128	64	2.50
22	MM23	none	64	≥128	8	0.19	≥128	64	1.50
23	MM26	none	64	≥128	2	0.05	≥128	64	1.50

[a]EC, *Escherichia coli*; KO, *Klebsiella oxytoca*; CF, *Citrobacter freundii;* EL, *Enterobacter cloacae*; KP, *Klebsiella pneumoniae*; MM, *Morganella morganii*. Additional β-lactamase determinants: b1: VIM-1, b2: IMP-4, b3: KPC-2, b4: CTX-M-3, b5: CTX-M-14, b6: CTX-M-15, b7: TEM-1, and b8: SHV-12. The synergistic effect is depicted by the FIC index, which is calculated as FIC (cpd) + FIC (Mem), where FIC (cpd) is the (MIC of cpd in combination with Mem)/(MIC of cpd alone) while FIC (Mem) is (MIC of cpd in combination with Mem)/(MIC of Mem alone). The drug combination is considered synergistic if the FIC Index ≤0.5. All experiments were performed in at least triplicates; the degree of inhibition of bacterial growth was determined with the naked eye after incubation.

*4. In vivo efficacy study of compound **11_a38** in combination with Mem*

To shed light on the potential clinical benefits of compound **11_a38**, evaluation of *in vivo* toxicity and antimicrobial efficacy was performed by employing a *Galleria mellonella* infection model. First, the tolerable dose of compound **11_a38** was investigated by injecting a solution of compound **11_a38** formulated in 50% PEG solution at different dosages (0, 10 or 30 mg/kg) into the hemocoel of larvae and the percent of survival were monitored at 12 h interval for 48 h. As shown in **Figure S51**, 100% survival rates were observed after 48 h for all treatment groups, indicating the relative safety of both the vehicle and the compound at the dosages tested. Next, the therapeutic abilities of compound **11_a38** alone, Mem alone or their combinations to protect the larvae against a lethal dose infection of clinical NDM-1-producing CRE isolate EL10 were determined at single doses of 0 or 10 mg/kg in drug monotherapy or 2 + 2 mg/kg, 10 + 10 mg/kg in drug combinations (**Figure 2**). 100% mortality of larvae were observed for the treatment groups of the vehicle, 10 mg/kg monotherapy of compound **11_a38**, and combination therapy at 2 + 2 mg/kg doses after 12 h. Encouragingly, 10 mg/kg monotherapy of Mem and combination

therapy at 10 + 10 mg/kg doses after 48 h resulted in 20% and 60% survival rate respectively, demonstrating the excellent ability of compound **11_a38** to potentiate Mem antibacterial activity against clinical NDM-1-producing CRE strains. Compared to the treatment groups of monotherapy of compound **11_a38** and Mem at 10 mg/kg, it was found to be highly significant ($p < 0.05$). These results suggest that the therapeutic efficacy of the combination of Mem and compound **11_a38** should be further evaluated in a mouse infection model in future.

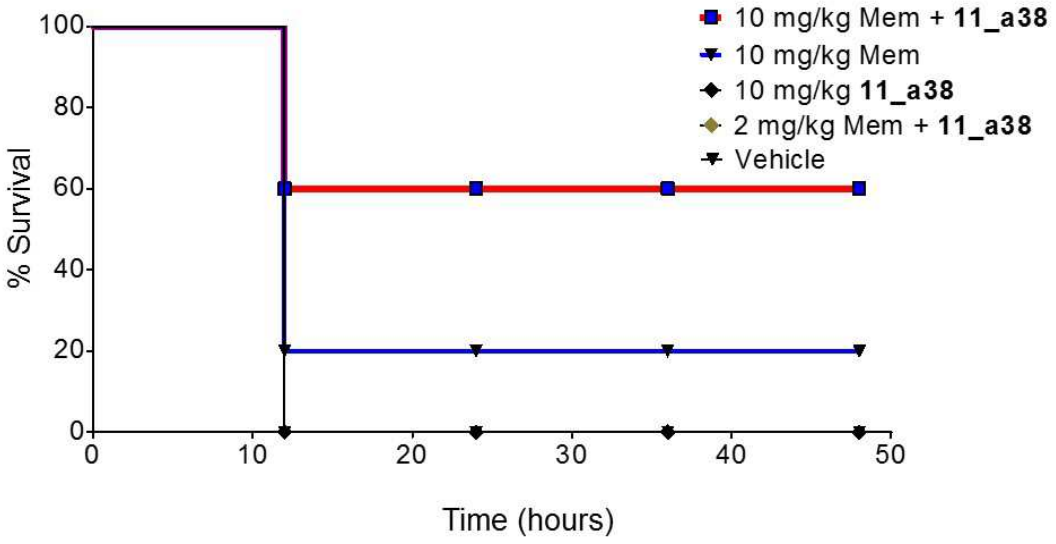


Figure 2. Kaplan-Meier survival analysis of monotherapy and combination therapy in protecting *Galleria mellonella* larvae infected with a lethal dose of the clinical NDM-1-producing CRE isolate EL10 (2.5 x 10^5 CFU/larva). Data are the means of three independent experiments.

*5. Biochemical studies of compound **11_a38** with purified NDM-1 protein*

To confirm whether the observed synergy is due to the inhibition of the NDM-1 activity of compound **11_38**, standard enzymatic assays using purified NDM-1 protein and colorimetric β-lactamase substrate nitrocefin were performed as previously described.[24] Compound **11_a38** demonstrated a potent *in vitro* dose-dependent inhibition of the NDM-1 enzyme, with a calculated IC_{50} value of about 13 µM (**Figure S49**). Previous studies of Eb indicated that it

inhibited the activity of NDM-1 in a time-dependent and concentration-dependent manner. Based on the similarity of the core structure of Eb and compound **11_38**, we reasoned that both compounds might exhibit similar NDM-1 inhibition mechanism. To test this hypothesis, the rapid high dilution method was performed to test the reversibility of inhibition of compound **11_a38**. The results revealed that approximately 40% residual NDM-1 activity was observed after the rapid high dilution of a 20-minutes pre-incubation mixture when compared to positive control with no addition of compound **11_a38** (**Figure S50**), implying that the inhibitory effect of compound **11_a38** most likely involves slowly reversible binding to the NDM-1 protein. Compared with Eb, which has 11% residual activity after the rapid high dilution, compound **11_a38** exhibited weaker binding to NDM-1. As illustrated in **Figure 3A**, inhibition of NDM-1 by compound **11_a38** was also time-dependent and concentration-dependent. Moreover, plots of natural logarithm of percentage residual activity versus incubation time at different concentrations of compound **11_a38** indicated that inhibition of DNM-1 follows *pseudo*-first order kinetics with calculated kinetic parameters K_I of 17 ± 2 μM, k_{inact} of 0.068 ± 0.007 min^{-1} and k_{inact}/K_I of 0.067 $mM^{-1}s^{-1}$ respectively (**Figure 3B**).

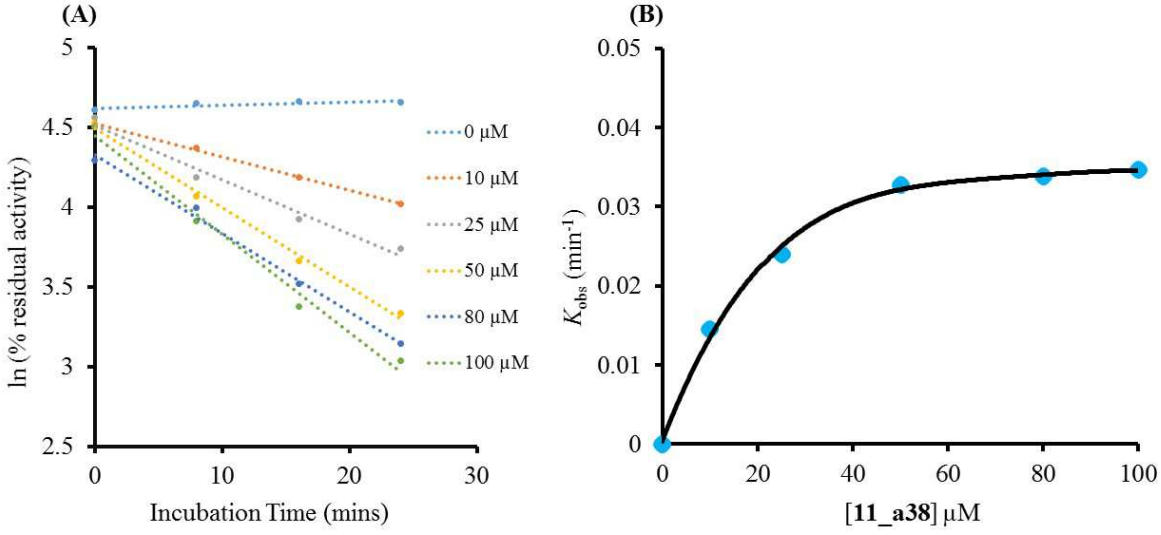


Figure 3. (A) Time-dependent and concentration-dependent inhibition of NDM-1 enzyme by compound **11_a38**; (B) Hyperbolic plot of K_{obs} of compound **11_a38** versus concentration of compound **11_a38**.

To better illustrate the nature of potential interaction between NDM-1 protein and compound **11_a38**, we examined the wild-type and denatured NDM-1 protein treated with compound **11_a38** respectively, using nano-ESI-MS. First, we obtained the ESI mass spectra of wild-type and denatured NDM-1 protein for comparison (**Figure 4**). The deconvoluted ESI mass spectra of wild-type and denatured NDM-1 protein revealed an intact mass of 25,995 Da and 25,866 Da respectively, with a difference of 129 Da, suggesting that there were two zinc ions (2 × 65 Da) in the wild-type NDM-1 protein. In contrast, the deconvoluted ESI-MS spectra of compound **11_a38**-treated wild-type and denatured NDM-1 protein revealed a complex where the majority of protein had a mass of 26,272 Da and 26,208 Da respectively, with a difference of 64 Da, indicating that there was only one zinc ion in the compound **11_a38**-treated wild-type NDM-1 protein. Since the molecular mass of compound **11_a38** is about 341 and there is only one cysteine present in the NDM-1 protein, the protein mass difference between the denatured NDM-1 protein and compound **11_a38**-treated denatured NDM-1 protein is 342 Da, indicating that only one covalent interaction event occurred between compound **11_a38** and a cysteine molecule. The major peak (26,272 Da) in the deconvoluted ESI-MS spectra of compound **11_a38**-treated wild-type NDM-1 protein, therefore, represented the sum of the molecular mass of a denatured NDM-1 protein (25,866 Da), compound **11_a38** (341 Da) and one zinc ion (65 Da). These results are consistent with those of the parental compound Eb.

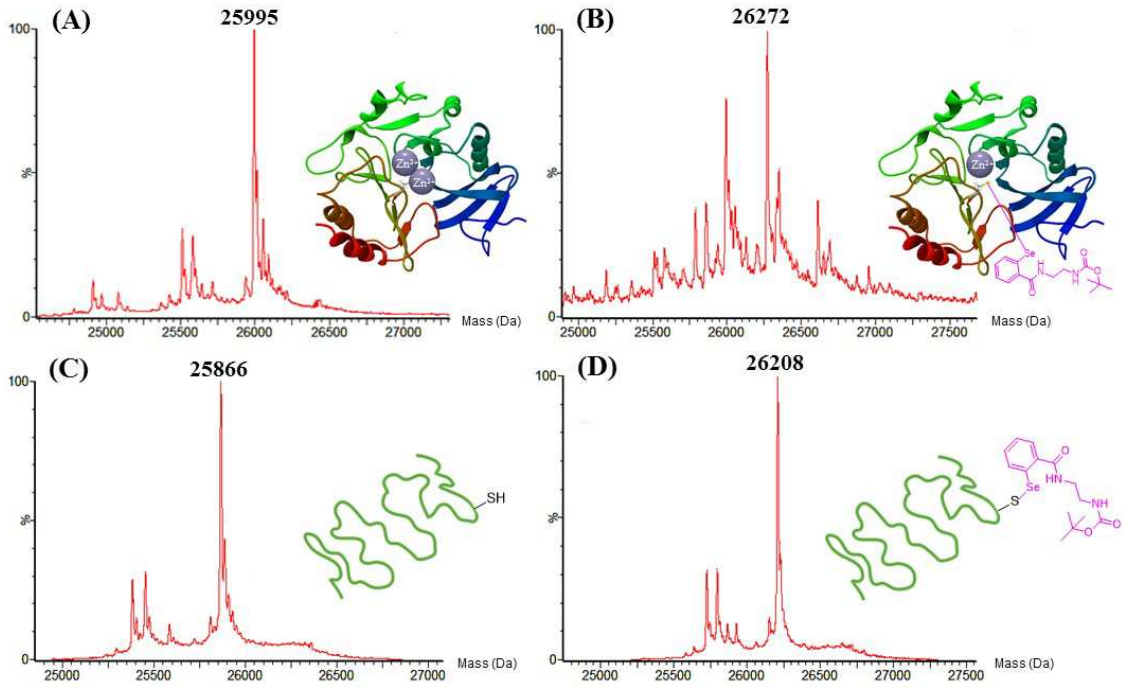


Figure 4. Nano-ESI-MS analysis and their cartoon representations of wild-type (A) and denatured (C) NDM-1 enzyme as well as compound **11_a38**-treated wild-type (B) and denatured (D) NDM-1 enzyme.

Based on the above data, the mechanism of inhibition of compound **11_a38** against NDM-1 protein was proposed. Both cationic divalent zinc ions in the active site of NDM-1 may act as Lewis acids to interact with the carbonyl oxygen of compound **11_a38**, causing the 1,2-benzisoselenazol-3(2*H*)-one being susceptible to nucleophilic attack by the nearby thiol group of cysteine. The thiol group of cysteine reacts with compound **11_a38** to form a new S-Se covalent bond, leading to the loss of one zinc ion and therefore inhibiting the NDM-1 activity.

*6. Cytotoxicity studies of compound **11_a38** against eukaryotic cells*

As mentioned before, overcoming compound toxicity has been a great concern for the development of clinically useful NDM-1 inhibitors. Although a recent report of phase 2 clinical

trial of Eb in an unrelated study indicated that treatment of Eb at a dose of 400 mg twice daily in human was safe,[37] a potential problem usually associated with cysteine modifying agents is their high toxicity towards eukaryotic cells. To verify the safety of compound **11_a38**, its toxicity was tested against human cervical cancer HeLa cell line and mouse peritoneal fibroblast L929 cell line respectively. As shown in **Figure 5**, compound **11_a38** exhibited relatively low cytotoxicity against Hela and L929 cell lines, with a cell viability of 70% or higher at a concentration of 2 mg/mL, which is the effective synergistic concentration in the combination study. Microscopic investigation of both cell lines also indicated that there were no morphological changes after incubation with compound **11_a38** at these concentrations, displaying negligible cytotoxicity.

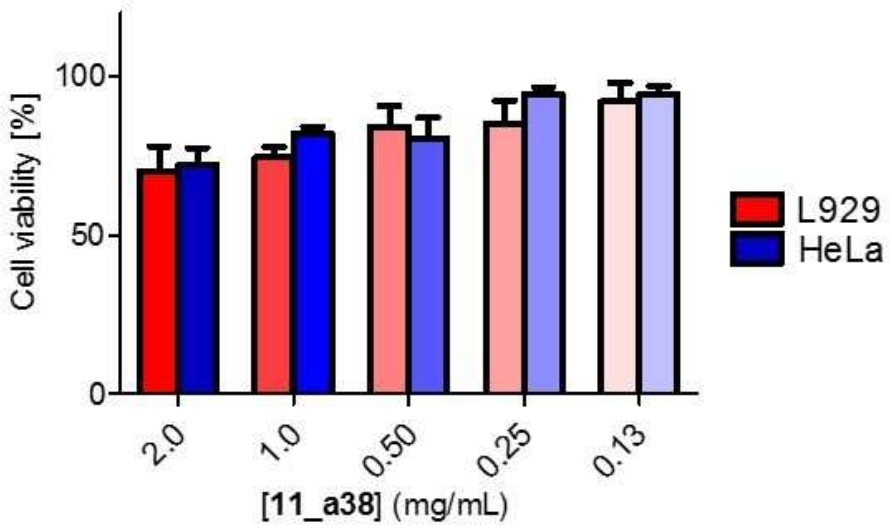


Figure 5. Cytotoxicity profile of compound **11_a38** against HeLa and L929 cell lines.

Conclusion

In this study, we demonstrated that structural modification of Eb through simple chemistry allows us to generate a focused compound library of 1,2-benzisoselenazol-3(2*H*)-one derivatives with forty-six candidates. Many compounds displayed stronger synergistic activity with Mem

(FIC index ≤0.5) than Eb as well as better physiochemical properties (cLogP < that of Eb) but negligible antibacterial activity (MIC ≥128 μg/mL) when tested alone. One of these compounds was also demonstrated to exhibit potent synergistic activities with Mem against a panel of clinical NDM-1-producing CRE isolates. These promising results led to the efficacy testing of the lead compound **11_a38** in the *Galleria mellonella* infection model. Investigation of the mode of action suggests that compound **11_a38** exerted synergistic antimicrobial effect with meropenem by covalently binding to the NDM-1 protein. In summary, because of its structural simplicity, potent synergistic activity in combination with Mem, and low cytotoxicity towards eukaryotic cells, a new class of 1,2-benzisoselenazol-3(2*H*)-one derivatives may represent excellent leads for the development of next-generation carbapenem adjuvant co-therapy.

Experimental section

Chemical Synthesis

All NMR spectra were recorded on a Bruker Advance-III spectrometer at 400 MHz for ^{1}H, 101 MHz for ^{13}C, and 376 MHz for ^{19}F. All NMR measurements were carried out at room temperature. The chemical shifts are reported as parts per million (ppm) in unit relative to the resonance of $CDCl_3$, DMSO-d_6, D_2O, Acetone-d_6, or MeOH-d_4. Mass spectra of low-resolution and high-resolution mass spectrometry were obtained on a Micromass Q-TOF-2 by electron spray ionization (ESI) mode. All chemical reagents and organic solvents were reagent grade and were used without further purification unless otherwise stated. The plates used for analytical thin-layer chromatography (TLC) were E. Merck Silica Gel $60F_{254}$ aluminum-backed plates (0.25 mm thickness) and were visualized under short and long UV light (254 and 365 nm) or stained with acidified potassium permanganate solution followed by gentle heating. Column

chromatographic purifications were carried out on MN silica gel 60 (230−400 mesh) with gradient elution. Compound purity was determined by an Agilent 1100 series HPLC installed with a normal phase Prep-Sil Scalar column (4.6 mm × 250 mm, 5 µm) at UV detection of 254 nm (reference at 450 nm). All tested compounds were shown to have >95% purity according to the HPLC. 3-Methyl-1-(((1,1,1-trifluoro-2-methylpropan-2-yl)oxy)carbonyl)-1*H*-imidazol-3-ium iodide was synthesized as described previously.[30] Amines **a20, a21**, **a25**, **a30**, **a31** and **a34** were prepared in two steps by mixing the corresponding acid chlorides, such as benzoyl chloride, 3,3-dimethylbutanoyl chloride, 4-methylbenzoyl chloride, 2,4-difluorobenzoyl chloride and thiophene-2-carbonyl chloride, or dimethyl chlorophosphate with amine **a38** respectively, followed by acidic treatment of TFA as previously reported.[31] Amines **a1**-**a19**, **a22**-**a24**, **a26**-**a29**, **a33**, **a35**-**a39**, anthranilic acid (**8**) and 3,3'-diselanediyldipropanoic acid (**9b**) are commercially available. Compound **11_a2**,[38] **11_a3**,[39] **11_a5**,[42] **11_a7**,[42] **11_a9**,[39] **11_a10**,[40] **11_a14**,[40] **11_a23**,[41] **11_a35**,[41] **11_a37**,[42] and **12**[26] have been reported in the literature previously. Other compounds reported here are new compounds.

2,2'-Diselanediyldibenzoic acid (9a)

Anthranilic acid (**8**) (18 g, 131 mmol) was dissolved in a solution of 37% hydrochloric acid (30 mL) and H_2O (30 mL). After the solution was cooled to 0 °C, a solution of $NaNO_2$ (10 g, 145 mmol in 10 mL H_2O) was added dropwise and the reaction mixture was stirred at 0 °C for 30 mins until all solid has been dissolved. The obtained diazonium salt was used immediately without further purification. To a well-stirred mixture of selenium powder (5.1 g, 65 mmol) in degassed H_2O (50 mL) under a nitrogen atmosphere, a $NaBH_4$ aqueous solution (4.45 g, 130 mmol in 10 mL H_2O) was added dropwise. After the Se powder has been completely dissolved,

another batch of 5.1 g Se powder was added to the solution in portions. The reaction mixture was heated for 30 mins to ensure all the Se powder has been dissolved completely. The obtained brownish-red solution was basified with NaOH (10 g) in an ice bath. After cooling the basified solution to 5 °C, the diazonium salt prepared above was added dropwise at a rate at which the reaction temperature was maintained below 10 °C. Afterwards, the mixture was stirred under the nitrogen atmosphere at 60 °C for 11 h and another 3 h at room temperature. The mixture was acidified with hydrochloric acid to pH = 3 ~ 4 and then filtered. The obtained brownish-red precipitates were re-dissolved in NaOH solution (pH = 9 ~ 10) and any undissolved black solid was removed by filtration. The brownish-red filtrate was re-acidified with hydrochloric acid to pH = 1 and the red precipitates were filtered and washed twice with water. The desired product was obtained by recrystallization in acetic acid as a light yellow solid (15.6 g) in 60% yield. ^{1}H NMR (400MHz, DMSO-d_6) δ 13.56 (br. s., 1 H), 8.04 (d, J = 6.4 Hz, 1 H), 7.68 (d, J = 7.6 Hz, 1 H), 7.49 (t, J = 7.6 Hz, 1 H), 7.36 (t, J = 7.0 Hz, 1 H); ^{13}C NMR (101MHz, DMSO-d_6) δ 169.0, 134.1, 134.0, 132.0, 130.0, 129.2, 127.0.

2-(Chlorocarbonyl)phenyl hypochloroselenoite (10)

To a solution of $SOCl_2$ (10 mL) in a three-necked round bottom flask under a nitrogen atmosphere at 0 °C, compound **9a** (2.0 g, 8 mmol) in portions and serval drops of DMF were added. The mixture was heated to reflux at 80 °C for 3 h. After heating, excess $SOCl_2$ was removed by blowing with nitrogen and 20 mL hexane were added to the flask to extract the product for three times. After evaporating the hexane under reduced pressure, a bright yellow product of compound **10** (2.0 g) was obtained in 80% yield. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.38

(d, *J* = 7.0 Hz, 1 H), 8.13 (d, *J* = 8.3 Hz, 1 H), 7.82 (t, *J* = 7.6 Hz, 1 H), 7.52 (t, *J* = 7.6 Hz, 1 H); ^{13}C NMR (101MHz, $CDCl_3$) δ 172.6, 146.3, 136.2, 134.6, 129.0, 127.1, 126.7.

2-(4-Chlorophenyl)benzo[*d*][1,2]selenazol-3(2*H*)-one (11_a2)

To a well-stirred solution of 4-chloroaniline (**a2**) (0.57 g, 4.5 mmol) and NEt_3 (5 mL) in dry DCM (25 mL) was added dropwise a solution of compound **10** (0.51 g, 2.0 mmol) at room temperature. The reaction mixture was stirred at room temperature for 14 h. The crude mixture was obtained by removal of the organic solvents under reduced pressure and was re-dissolved in DCM, followed by washing with H_2O for 3 times. The organic layers were combined, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuum. Purification of the product was performed by flash column chromatography on silica gel to afford the desired compound **11_a2** (0.28 g) in 45% yield. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.04 (d, *J* = 7.8 Hz, 1 H), 7.59 (d, *J* = 3.9 Hz, 2 H), 7.51 (d, *J* = 1.0 Hz, 2 H), 7.41 (t, *J* = 3.9, 7.8 Hz, 1 H), 7.32 (d, *J* = 1.0 Hz, 2 H); ^{13}C NMR (101MHz, $CDCl_3$) δ 185.4, 134.7, 134.0, 129.4, 128.1, 126.7, 126.6, 125.2, 123.8, 121.0; HRMS *m*/*z* calcd for $(C_{13}H_8ClNOSe + H)^+$ 309.9532, found 309.9531.

2-(4-Methoxyphenyl)benzo[*d*][1,2]selenazol-3(2*H*)-one (11_a3)

Following the experimental procedure for the preparation of compound **11_a2** described above, but with 4-methoxyaniline (**a3**) (0.55 g, 4.5 mmol) as a starting material, compound **11_a3** (0.32 g) was obtained in 52% yield. ^{1}H NMR (400MHz, DMSO-d_6) δ 8.08 (d, *J* = 7.8 Hz, 1 H), 7.89 (d, *J* = 7.8 Hz, 1 H), 7.67 (t, *J* = 1.0 Hz, 1 H), 7.53 - 7.45 (m, 3 H), 7.01 (d, *J* = 8.8 Hz, 2 H), 3.79 (s, 3 H); ^{13}C NMR (101MHz, DMSO-d_6) δ 165.4, 157.8, 139.5, 132.7, 132.5, 128.8, 128.3,

127.0, 126.6, 126.3, 114.8, 55.9; HRMS *m*/*z* calcd for $(C_{14}H_{11}NO_2Se + H)^+$ 306.0028, found 306.0033.

2-(4-Isopropylphenyl)benzo[*d*][1,2]selenazol-3(2*H*)-one (11_a4)

Following the experimental procedure for the preparation of compound **11_a2** described above, but with 4-isopropylaniline (**a4**) (0.61 g, 4.5 mmol) as a starting material, compound **11_a4** (0.35 g) was obtained in 55% yield. ^{1}H NMR (400MHz, DMSO-d_6) δ 8.09 (d, *J* = 7.8 Hz, 1 H), 7.90 (d, *J* = 7.8 Hz, 1 H), 7.68 (t, *J* = 7.8 Hz, 1 H), 7.53 (d, *J* = 1.0 Hz, 2 H), 7.48 (t, *J* = 1.0 Hz, 1 H), 7.33 - 7.30 (m, *J* = 7.8 Hz, 2 H), 2.91 (d, *J* = 6.8 Hz, 1 H), 1.22 (d, *J* = 6.8 Hz, 6 H); ^{13}C NMR (101MHz, DMSO-d_6) δ 165.4, 146.6, 139.4, 137.8, 132.6, 129.0, 128.4, 127.5, 126.7, 126.3, 125.3, 33.5, 24.3; HRMS *m*/*z* calcd for $(C_{16}H_{15}NOSe + H)^+$ 318.0392, found 318.0396.

3-(3-Oxobenzo[*d*][1,2]selenazol-2(3*H*)-yl)benzoic acid (11_a5)

Following the experimental procedure for the preparation of compound **11_a2** described above, but with 3-aminobenzoic acid (**a5**) (0.62 g, 0.45 mmol) as a starting material, compound **11_a5** (0.19 g) was obtained in 30% yield. ^{1}H NMR (400MHz, DMSO-d_6) δ 13.14 (br. s., 1 H), 8.25 (s, 1 H), 8.11 (d, *J* = 8.3 Hz, 1 H), 7.93 (d, *J* = 7.6 Hz, 1 H), 7.89 (d, *J* = 8.3 Hz, 1 H), 7.84 (d, *J* = 7.6 Hz, 1 H), 7.71 (t, *J* = 7.3 Hz, 1 H), 7.60 (t, *J* = 1.0 Hz, 1 H), 7.50 (t, *J* = 7.6 Hz, 1 H); ^{13}C NMR (101MHz, DMSO-d_6) δ 167.3, 165.7, 140.5, 139.3, 132.9, 132.2, 130.1, 129.2, 128.8, 128.5, 126.9, 126.8, 126.4, 125.4; HRMS *m*/*z* calcd for $(C_{14}H_9NO_3Se + H)^+$ 319.9820, found 319.9822.

2-(4-(Hydroxymethyl)phenyl)benzo[*d*][1,2]selenazol-3(2*H*)-one (11_a6)

Following the experimental procedure for the preparation of compound **11_a2** described above, but with 4-aminobenzyl alcohol (**a6**) (0.55 g, 4.5 mmol) as a starting material, compound **11_a6** (0.25 g) was obtained in 40% yield. ^{1}H NMR (400MHz, DMSO-d_6) δ 8.11 (d, J = 8.3 Hz, 1 H), 7.91 (d, J = 7.6 Hz, 1 H), 7.69 (t, J = 7.3 Hz, 1 H), 7.59 (d, J = 1.0 Hz, 2 H), 7.49 (t, J = 7.6 Hz, 1 H), 7.40 (d, J = 1.0 Hz, 2 H), 5.25 (t, J = 5.4 Hz, 1 H), 4.53 (d, J = 5.1 Hz, 2 H); ^{13}C NMR (101MHz, DMSO-d_6) δ 165.4, 140.7, 139.4, 138.7, 132.6, 129.0, 128.4, 127.7, 126.7, 126.3, 124.9, 62.9; HRMS *m*/*z* calcd for $(C_{14}H_{11}NO_2Se + H)^+$ 306.0028, found 306.0030.

4-(3-Oxobenzo[*d*][1,2]selenazol-2(3*H*)-yl)benzoic acid (11_a7)

Following the experimental procedure for the preparation of compound **11_a2** described above, but with 4-aminobenzoic acid (**a7**) (0.62 g, 4.5 mmol) as a starting material, compound **11_a7** (0.24 g) was obtained in 38% yield. ^{1}H NMR (400MHz, DMSO-d_6) δ 8.10 (d, J = 7.8 Hz, 1 H), 8.01 (d, J = 7.8 Hz, 2 H), 7.93 (d, J = 7.8 Hz, 1 H), 7.85 (d, J = 8.8 Hz, 2 H), 7.70 (t, J = 6.8 Hz, 1 H), 7.49 (t, J = 1.0 Hz, 1 H); ^{13}C NMR (101MHz, DMSO-d_6) δ 165.8, 139.2, 133.1, 131.0, 129.1, 128.6, 126.9, 126.4, 123.9; HRMS *m*/*z* calcd for $(C_{14}H_9NO_3Se + H)^+$ 319.9820, found 319.9825.

2-(3-Chloro-4-methoxyphenyl)benzo[*d*][1,2]selenazol-3(2*H*)-one (11_a8)

Following the experimental procedure for preparation of compound **11_a2** as described above but with 3-chloro-4-methoxyaniline (**a8**) (0.71 g, 4.5 mmol) as a starting material, compound **11_a8** (0.28 g) was obtained in 41% yield. ^{1}H NMR (400MHz, DMSO-d_6) δ 8.08 (br. s., 1 H), 7.90 (br. s., 1 H), 7.77 (br. s., 1 H), 7.69 (br. s., 1 H), 7.48 (br. s., 2 H), 7.23 (br. s., 1 H), 3.89 (br. s., 3 H); ^{13}C NMR (101MHz, DMSO-d_6) δ 165.6, 153.1, 139.5, 133.3, 132.7, 128.6, 128.4,

126.9, 126.7, 126.3, 125.4, 121.4, 113.5, 56.8; HRMS *m/z* calcd for $(C_{14}H_{10}ClNO_2Se + H)^+$ 339.9638, found 339.9639.

2-Benzylbenzo[*d*][1,2]selenazol-3(2*H*)-one (11_a9)

Following the experimental procedure for the preparation of compound **11_a2** described above but with benzyl amine (**a9**) (0.49 g, 4.5 mmol) as a starting material, compound **11_a9** (0.35 g) was obtained in 60% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, *J* = 7.83 Hz, 1H), 7.59 (d, *J* = 3.91 Hz, 2H), 7.42 - 7.49 (m, 1H), 7.38 (s, 5H), 5.04 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.2, 138.1, 137.2, 132.0, 129.0, 128.9, 128.6, 128.3, 127.4, 126.2, 124.0, 48.7; HRMS *m/z* calcd for $(C_{14}H_{11}NOSe + H)^+$ 290.0079, found 290.0085.

2-(4-Methoxybenzyl)benzo[*d*][1,2]selenazol-3(2*H*)-one (11_a10)

Following the experimental procedure for the preparation of compound **11_a2** described above but with 4-methoxybenzyl amine (**a10**) (0.62 g, 4.5 mmol) as a starting material, compound **11_a10** (0.31 g) was obtained in 45% yield. ^{1}H NMR (400MHz, DMSO-d_6) δ 7.97 (d, *J* = 7.8 Hz, 1 H), 7.82 (d, *J* = 7.8 Hz, 1 H), 7.57 (t, *J* = 7.8 Hz, 1 H), 7.43 - 7.37 (m, 1 H), 7.26 (d, *J* = 8.8 Hz, 2 H), 6.89 (d, *J* = 7.8 Hz, 2 H), 4.81 (s, 2 H), 3.71 (s, 3 H); ^{13}C NMR (101MHz, DMSO-d_6) δ 166.6, 159.2, 139.7, 132.0, 130.7, 130.1, 128.5, 127.9, 126.3, 114.4, 55.6, 46.8; HRMS *m/z* calcd for $(C_{15}H_{13}NO_2Se + Na)^+$ 342.0010, found 342.0010.

2-(3-(Piperidin-1-yl)benzyl)benzo[*d*][1,2]selenazol-3(2*H*)-one (11_a11)

Following the experimental procedure for the preparation of compound **11_a2** described above but with 3-(1-piperidinyl)benzyl amine (**a11**) (0.86 g, 4.5 mmol) as a starting material,

compound **11_a11** (0.07 g) was obtained in 10% yield. ^{1}H NMR (400MHz, MeOH-d_4) δ 7.97 (d, *J* = 7.8 Hz, 1 H), 7.88 (d, *J* = 7.8 Hz, 1 H), 7.60 (t, *J* = 7.3 Hz, 1 H), 7.45 (t, *J* = 1.0 Hz, 1 H), 7.22 (t, *J* = 1.0 Hz, 1 H), 6.98 (s, 1 H), 6.93 (d, *J* = 7.8 Hz, 1 H), 6.81 (d, *J* = 6.8 Hz, 1 H), 4.93 (s, 2 H), 3.13 (t, *J* = 4.9 Hz, 4 H), 1.71 - 1.65 (m, *J* = 5.4, 5.4 Hz, 4 H), 1.59 - 1.54 (m, 2 H); ^{13}C NMR (101MHz, MeOH-d_4) δ 167.8, 152.6, 139.8, 138.1, 131.7, 129.1, 127.6, 127.5, 125.8, 124.8, 119.2, 116.5, 116.4, 50.6, 25.4, 23.9; HRMS *m*/*z* calcd for $(C_{19}H_{20}N_2OSe + H)^+$ 373.0814, found 373.0817.

2-([1,1'-Biphenyl]-3-ylmethyl)benzo[*d*][1,2]selenazol-3(2*H*)-one (11_a12)

Following the experimental procedure for the preparation of compound **11_a2** described above but with 3-phenylbenzyl amine (**a12**) (0.82 g, 4.5 mmol) as a starting material, compound **11_a12** (0.58 g) was obtained in 80% yield. ^{1}H NMR (400MHz, MeOH-d_4) δ 7.98 (d, *J* = 7.8 Hz, 1 H), 7.89 (d, *J* = 8.8 Hz, 1 H), 7.62 - 7.55 (m, 5 H), 7.47 - 7.39 (m, 4 H), 7.33 (d, *J* = 7.8 Hz, 2 H), 5.06 (s, 2 H); ^{13}C NMR (101MHz, MeOH-d_4) δ 167.5, 141.7, 140.6, 139.8, 138.1, 131.7, 129.0, 128.5, 127.6, 127.5, 127.2, 126.9, 126.6, 126.6, 126.4, 125.8, 124.9; HRMS *m*/*z* calcd for $(C_{20}H_{15}NOSe + H)^+$ 366.0393, found 366.0395.

2-(Pyridin-4-yl)benzo[*d*][1,2]selenazol-3(2*H*)-one (11_a13)

Following the experimental procedure for the preparation of compound **11_a2** described above but with 4-aminopyridine (**a13**) (0.41 g, 4.5 mmol) as a starting material, compound **11_a13** (0.26 g) was obtained in 48% yield. ^{1}H NMR (400MHz, DMSO-d_6) δ 8.56 (d, *J* = 4.9 Hz, 2 H), 8.08 (d, *J* = 7.8 Hz, 1 H), 7.93 (d, *J* = 6.8 Hz, 1 H), 7.83 (d, *J* = 4.9 Hz, 2 H), 7.71 (t, *J* = 7.3 Hz, 1 H), 7.49 (t, *J* = 7.3 Hz, 1 H); ^{13}C NMR (101MHz, DMSO-d_6) δ 166.5, 151.2, 147.8, 138.8,

133.5, 129.2, 128.6, 127.0, 126.4, 117.3; HRMS *m*/*z* calcd for $(C_{12}H_8NO_2Se + H)^+$ 276.9875, found 276.9881.

2-Cyclohexylbenzo[*d*][1,2]selenazol-3(2*H*)-one (11_a14)

Following the experimental procedure for the preparation of compound **11_a2** described above but with cyclohexylamine (**a14**) (0.45 g, 4.5 mmol) as a starting material, compound **11_a14** (0.25 g) was obtained in 44% yield. ^{1}H NMR (400MHz, DMSO-d_6) δ 8.05 (d, *J* = 7.8 Hz, 1 H), 7.81 (d, *J* = 7.8 Hz, 1 H), 7.59 (t, *J* = 1.0 Hz, 1 H), 7.41 (t, *J* = 1.0 Hz, 1 H), 4.23 (br. s., 1 H), 1.92 (d, *J* = 9.8 Hz, 2 H), 1.80 (d, *J* = 11.7 Hz, 2 H), 1.64 (d, *J* = 12.7 Hz, 1 H), 1.49 - 1.33 (m, 4 H), 1.25 - 1.14 (m, 1 H); ^{13}C NMR (101MHz, DMSO-d_6) δ 166.1, 139.4, 131.7, 129.3, 127.7, 126.3, 126.2, 53.2, 34.0, 25.7, 25.3; HRMS *m*/*z* calcd for $(C_{13}H_{15}NOSe + H)^+$ 282.0392, found 282.0395.

2-(3-Methoxybenzoyl)benzo[*d*][1,2]selenazol-3(2*H*)-one (11_a15)

Following the experimental procedure for the preparation of compound **11_a2** described above but with 3-methoxybenzamide (**a15**) (0.68 g, 4.5 mmol) as a starting material, compound **11_a15** (0.37 g) was obtained in 55% yield. ^{1}H NMR (400MHz, DMSO-d_6) δ 8.05 (d, *J* = 8.3 Hz, 1 H), 7.83 (d, *J* = 7.6 Hz, 1 H), 7.75 (t, *J* = 7.6 Hz, 1 H), 7.46 (t, *J* = 7.0 Hz, 1 H), 7.38 (t, *J* = 8.3 Hz, 1 H), 7.24 - 7.16 (m, 2 H), 7.14 (d, *J* = 8.3 Hz, 1 H), 3.79 (s, 3 H); ^{13}C NMR (101MHz, DMSO-d_6) δ 171.1, 164.9, 159.1, 139.9, 136.4, 134.8, 129.8, 129.5, 129.1, 127.0, 126.6, 121.0, 117.5, 114.1, 55.8; HRMS *m*/*z* calcd for $(C_{15}H_{11}NO_3Se + Na)^+$ 355.9796, found 355.9806.

2-((4-Chlorophenyl)sulfonyl)benzo[*d*][1,2]selenazol-3(2*H*)-one (11_a16)

Following the experimental procedure for the preparation of compound **11_a2** described above but with 4-chlorobenzenesulfonamide (**a16**) (0.86 g, 4.5 mmol) as a starting material, compound **11_a16** (0.35 g) was obtained in 47% yield. ^{1}H NMR (400MHz, Acetone-d_6) δ 8.06 (d, *J* = 8.8 Hz, 2 H), 7.89 (d, *J* = 7.8 Hz, 1 H), 7.77 (d, *J* = 7.8 Hz, 1 H), 7.67 (d, *J* = 1.0 Hz, 1 H), 7.62 (d, *J* = 8.8 Hz, 2 H), 7.40 (t, *J* = 7.3 Hz, 1 H); ^{13}C NMR (101MHz, Acetone-d_6) δ 158.5, 133.8, 130.7, 128.3, 124.3, 123.3, 122.8, 120.9, 120.8, 119.4; HRMS *m*/*z* calcd for $(C_{13}H_8ClNO_3SSe + Na)^+$ 395.8971, found 395.8972.

2-(2-Morpholinoethyl)benzo[*d*][1,2]selenazol-3(2*H*)-one (11_a17)

Following the experimental procedure for the preparation of compound **11_a2** described above but with 4-(2-aminoethyl)morpholine (**a17**) (0.54 g, 4.5 mmol) as a starting material, compound **11_a17** (0.21 g) was obtained in 33% yield. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.94 (d, *J* = 7.8 Hz, 1 H), 7.59 (d, *J* = 7.8 Hz, 1 H), 7.45 (t, *J* = 7.8 Hz, 1 H), 7.28 (t, *J* = 7.3 Hz, 1 H), 3.95 - 3.85 (m, 2 H), 3.81 - 3.66 (m, 5 H), 2.53 (br. s., 6 H); ^{13}C NMR (101MHz, MeOH-d_4) δ 168.4, 142.8, 131.4, 127.3, 127.0, 125.5, 124.5, 66.4, 57.1, 53.0, 40.4; HRMS *m*/*z* calcd for $(C_{13}H_{16}N_2O_2Se + H)^+$ 313.0450, found 313.0460.

(*S*)-2-((1-ethylpyrrolidin-2-yl)methyl)benzo[*d*][1,2]selenazol-3(2*H*)-one (11_a18)

Following the experimental procedure for the preparation of compound **11_a2** described above but with (*S*)-(−)-2-aminomethyl-1-ethylpyrrolidine (**a18**) (0.58 g, 4.5 mmol) as a starting material, compound **11_a18** (0.34 g) was obtained in 55% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, *J* = 7.82 Hz, 1H), 7.60 (d, *J* = 7.82 Hz, 1H), 7.55 (t, *J* = 7.34 Hz, 1H), 7.38 (t, *J* = 7.34 Hz, 1H), 4.60 (d, *J* = 13.69 Hz, 1H), 3.42 (dd, *J* = 1.96, 13.69 Hz, 1H), 3.31 (t, *J* = 6.85 Hz, 1H),

2.95 - 3.08 (m, 1H), 2.83 - 2.92 (m, 1H), 2.36 - 2.45 (m, 1H), 2.27 - 2.35 (m, 1H), 1.67 - 1.96 (m, 4H), 1.27 (t, *J* = 7.34 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.9, 143.8, 131.4, 128.0, 127.7, 125.5, 123.3, 62.1, 52.8, 48.4, 44.6, 27.2, 23.3, 13.5; HRMS *m/z* calcd for $(C_{14}H_{18}N_2OSe + H)^+$ 312.0652, found 312.0654.

(*R*)-2-((1-ethylpyrrolidin-2-yl)methyl)benzo[*d*][1,2]selenazol-3(2*H*)-one (11_a19)

Following the experimental procedure for the preparation of compound **11_a2** described above but with (*R*)-(+)-2-aminomethyl-1-ethylpyrrolidine (**a19**) (0.58 g, 4.5 mmol) as a starting material, compound **11_a19** (0.33 g) was obtained in 53% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, *J* = 7.82 Hz, 1H), 7.55 - 7.63 (m, 1H), 7.47 - 7.55 (m, 1H), 7.31 - 7.39 (m, 1H), 4.52 - 4.63 (m, 1H), 3.39 (dd, *J* = 2.93, 14.67 Hz, 1H), 3.27 (t, *J* = 7.34 Hz, 1H), 2.97 (dd, *J* = 7.34, 12.23 Hz, 1H), 2.79 - 2.89 (m, 1H), 2.32 - 2.41 (m, 1H), 2.23 - 2.32 (m, 1H), 1.82 - 1.94 (m, 1H), 1.63 - 1.82 (m, 3H), 1.24 (t, *J* = 7.34 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.9, 143.8, 131.4, 127.9, 127.6, 125.4, 123.3, 62.1, 52.8, 48.3, 44.6, 27.2, 23.3, 13.5; HRMS *m/z* calcd for $(C_{14}H_{18}N_2OSe + H)^+$ 312.0659, found 312.0657.

***N*-(2-(3-oxobenzo[*d*][1,2]selenazol-2(3*H*)-yl)ethyl)benzamide (11_a20)**

Following the experimental procedure for the preparation of compound **11_a2** described above but with *N*-(2-aminoethyl)benzamide (**a20**) (0.74 g, 4.5 mmol) as a starting material, compound **11_a20** (89 mg) was obtained in 13% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (t, *J* = 4.89 Hz, 1H), 8.01 (d, *J* = 7.82 Hz, 1H), 7.81 - 7.88 (m, 3H), 7.60 (t, *J* = 6.85 Hz, 1H), 7.52 (d, *J* = 6.85 Hz, 1H), 7.47 (d, *J* = 7.83 Hz, 2H), 7.41 - 7.45 (m, 1H), 3.91 (t, *J* = 5.87 Hz, 2H), 3.49 - 3.59 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.3, 167.2, 140.1, 134.7, 132.1, 131.8, 128.8, 128.8,

128.0, 127.8, 127.6, 127.6, 126.3, 126.1, 43.3; HRMS *m/z* calcd for $(C_{16}H_{14}N_2O_2Se + H)^+$ 347.0293, found 347.0294.

3,3-Dimethyl-*N*-(2-(3-oxobenzo[*d*][1,2]selenazol-2(3*H*)-yl)ethyl)butanamide (11_a21)

Following the experimental procedure for the preparation of compound **11_a2** described above but with *N*-(2-aminoethyl)-3,3-dimethylbutanamide (**a21**) (0.71 g, 4.5 mmol) as starting material, compound **11_a21** (0.18 g) was obtained in 27% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 7.82 Hz, 1H), 7.70 (d, *J* = 7.82 Hz, 1H), 7.61 (t, *J* = 7.34 Hz, 1H), 7.38 - 7.49 (m, 1H), 6.51 (br. s., 1H), 4.00 (t, *J* = 5.87 Hz, 2H), 3.52 - 3.66 (m, 2H), 2.06 (s, 2H), 0.98 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.4, 168.2, 138.3, 132.2, 128.7, 126.8, 126.3, 124.2, 50.4, 44.2, 40.3, 30.8, 29.8; HRMS *m/z* calcd for $(C_{15}H_{20}N_2O_2Se + H)^+$ 341.0763, found 341.0766.

2-(2-(Piperidin-1-yl)ethyl)benzo[*d*][1,2]selenazol-3(2*H*)-one (11_a22)

Following the experimental procedure for the preparation of compound **11_a2** described above but with 1-(2-aminoethyl)piperidine (**a22**) (0.58 g, 4.5 mmol) as a starting material, compound **11_a22** (0.32 g) was obtained in 52% yield. ^{1}H NMR (400MHz, MeOH-d_4) δ 7.90 (d, *J* = 7.8 Hz, 1 H), 7.93 (d, *J* = 7.8 Hz, 1 H), 7.58 (t, *J* = 7.3 Hz, 1 H), 7.40 (t, *J* = 1.0 Hz, 1 H), 3.95 (t, *J* = 5.4 Hz, 2 H), 2.65 - 2.51 (m, 6 H), 1.73 (t, *J* = 1.0 Hz, 4 H); ^{13}C NMR (101MHz, MeOH-d_4) δ 168.3, 131.3, 127.4, 126.9, 126.9, 125.4, 124.4, 57.3, 54.0, 40.9, 25.5, 24.0; HRMS *m/z* calcd for $(C_{14}H_{18}N_2OSe + H)^+$ 311.0717, found 311.0659.

2-(2-(Dimethylamino)ethyl)benzo[*d*][1,2]selenazol-3(2*H*)-one (11_a23)

Following the experimental procedure for the preparation of compound **11_a2** described above but with *N*,*N*-dimethylethylenediamine (**a23**) (0.40 g, 4.5 mmol) as a starting material, compound **11_a23** (0.22 g) was obtained in 41% yield. ^{1}H NMR (400MHz, MeOH-d_4) δ 7.93 (d, *J* = 7.8 Hz, 1 H), 7.86 (d, *J* = 7.8 Hz, 1 H), 7.56 (t, *J* = 7.8 Hz, 1 H), 7.39 (t, *J* = 1.0 Hz, 1 H), 3.93 (t, *J* = 5.9 Hz, 2 H), 2.62 (t, *J* = 5.9 Hz, 2 H), 2.34 (s, 7 H); ^{13}C NMR (101MHz, MeOH-d_4) δ 168.5, 133.9, 132.1, 122.7, 66.3, 55.9, 53.2, 34.2; HRMS *m/z* calcd for $(C_{11}H_{14}N_2OSe + H)^+$ 271.0344, found 271.0351.

2-(2-(Diethylamino)ethyl)benzo[*d*][1,2]selenazol-3(2*H*)-one (11_a24)

Following the experimental procedure for the preparation of compound **11_a2** described above but with *N*,*N*-diethylethylenediamine (**a24**) (0.52 g, 4.5 mmol) as a starting material, compound **11_a24** (0.38 g) was obtained in 64% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, *J* = 7.82 Hz, 1H), 7.59 (d, *J* = 7.82 Hz, 1H), 7.49 (t, *J* = 7.83 Hz, 1H), 7.26 - 7.39 (m, 1H), 3.87 - 4.01 (m, 2H), 2.61 - 2.76 (m, 6H), 1.07 (t, *J* = 6.85 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.9, 143.1, 131.3, 127.9, 127.6, 125.5, 123.5, 52.6, 45.7, 41.5, 10.8; HRMS *m/z* calcd for $(C_{13}H_{18}N_2OSe + H)^+$ 300.0652, found 300.0654.

4-Methyl-*N*-(2-(3-oxobenzo[*d*][1,2]selenazol-2(3*H*)-yl)ethyl)benzamide (11_a25)

Following the experimental procedure for the preparation of compound **11_a2** described above but with *N*-(2-aminoethyl)-4-methylbenzamide (**a25**) (0.80 g, 4.5 mmol) as a starting material, compound **11_a25** (0.39 g) was obtained in 55% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.52 - 8.70 (m, 1H), 8.02 (d, *J* = 7.83 Hz, 1H), 7.84 (d, *J* = 7.83 Hz, 1H), 7.71 - 7.81 (m, *J* = 7.82 Hz, 2H), 7.60 (t, *J* = 7.82 Hz, 1H), 7.36 - 7.49 (m, 1H), 7.20 - 7.33 (m, *J* = 7.82 Hz, 2H), 3.91 (t, *J* =

5.38 Hz, 2H), 3.53 (d, *J* = 5.87 Hz, 2H), 2.35 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.0, 166.8, 141.5, 140.2, 132.1, 131.9, 129.3, 128.2, 127.8, 127.7, 126.3, 126.2, 43.3, 21.4; HRMS *m/z* calcd for $(C_{17}H_{16}N_2O_2Se + H)^+$ 361.0450, found 361.0456.

2-(2-(Benzylamino)ethyl)benzo[*d*][1,2]selenazol-3(2*H*)-one (11_a26)

Following the experimental procedure for the preparation of compound **11_a2** described above but with N^1-benzylethane-1,2-diamine (**a26**) (0.68 g, 4.5 mmol) as a starting material, compound **11_a26** (0.07 g) was obtained in 11% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, *J* = 7.82 Hz, 1H), 7.64 (d, *J* = 7.82 Hz, 1H), 7.57 (t, *J* = 7.34 Hz, 1H), 7.33 - 7.47 (m, 5H), 7.25 - 7.33 (m, 1H), 3.94 - 4.06 (m, 2H), 3.90 (s, 2H), 2.91 - 3.07 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.9, 141.0, 139.6, 131.7, 128.6, 128.3, 128.2, 127.4, 127.2, 125.9, 123.7, 53.6, 48.7, 44.1; HRMS *m/z* calcd for $(C_{16}H_{16}N_2OSe + H)^+$ 333.0501, found 333.0504.

2-(2-(2,6-Dimethylmorpholino)ethyl)benzo[*d*][1,2]selenazol-3(2*H*)-one (11_a27)

Following the experimental procedure for the preparation of compound **11_a2** described above but with 2-(2,6-dimethylmorpholin-4-yl)ethanamine (**a27**) (0.71 g, 4.5 mmol) as a starting material, compound **11_a28** (0.22 g) was obtained in 32% yield. ^{1}H NMR (400MHz, MeOH-d_4) δ 7.91 (d, *J* = 7.8 Hz, 1 H), 7.94 (d, *J* = 7.8 Hz, 1 H), 7.60 (t, *J* = 6.8 Hz, 1 H), 7.42 (t, *J* = 7.3 Hz, 1 H), 4.00 (t, *J* = 1.0 Hz, 2 H), 3.95 - 3.86 (m, 2 H), 2.96 (d, *J* = 10.8 Hz, 2 H), 2.70 - 2.65 (m, 2 H), 1.90 (t, *J* = 11.2 Hz, 2 H), 1.18 (d, *J* = 5.9 Hz, 6 H); ^{13}C NMR (101MHz, MeOH-d_4) δ 158.0, 143.0, 131.4, 126.9, 125.4, 124.4, 121.0, 71.6, 58.7, 56.7, 40.5, 17.9; HRMS *m/z* calcd for $(C_{15}H_{20}N_2O_2Se + H)^+$ 341.0763, found 341.0770.

(*R*)-2-((*R*)-2-(3-oxobenzo[*d*][1,2]selenazol-2(3*H*)-yl)propanamido)propanoic acid (11_a28)

A solution of compound **10** (0.51 g, 2 mmol) in DCM was added dropwise into the solution of D-Ala-D-Ala (**a28**) (0.72 g, 4.5 mmol) in acetonitrile. The mixture was stirred under room temperature for 48 hours. After the reaction was completed, acetonitrile was removed under reduced pressure. The oily residue was dissolved in water and the product was extracted by EA and purified by column chromatography on silica gel with DCM and EA/MeOH (10:1) as eluent to afford the title compound **11_a28** (0.03 g) in 5% yield. ^{1}H NMR (400MHz, MeOH-d_4) δ 7.94 (t, *J* = 9.3 Hz, 2 H), 7.62 (t, *J* = 7.8 Hz, 1 H), 7.44 (t, *J* = 1.0 Hz, 1 H), 5.32 - 5.25 (m, 1 H), 4.31 - 4.16 (m, 1 H), 1.52 (d, *J* = 6.8 Hz, 3 H), 1.36 (d, *J* = 7.8 Hz, 3 H); ^{13}C NMR (101MHz, MeOH-d_4) δ 178.0, 170.5, 168.0, 141.5, 131.6, 127.3, 127.2, 125.5, 124.6, 53.0, 50.7, 17.8; HRMS *m*/*z* calcd for $(C_{13}H_{14}N_2O_4Se + H)^+$ 343.0192, found 343.0197.

2-(5-(Diethylamino)pentan-2-yl)benzo[*d*][1,2]selenazol-3(2*H*)-one (11_a29)

Following the experimental procedure for preparation of compound **11_a2** described above but with N^1,N^1-diethylpentane-1,4-diamine (**a29**) (0.72 g, 4.5 mmol) as a starting material, compound **11_a29** (0.33 g) was obtained in 48% yield. ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.97 (d, *J* = 6.85 Hz, 2H), 7.59 (t, *J* = 7.34 Hz, 1H), 7.44 (t, *J* = 7.34 Hz, 1H), 4.61 - 4.77 (m, 1H), 2.31 - 2.59 (m, 6H), 1.67 (d, *J* = 6.85 Hz, 2H), 1.41 - 1.57 (m, 2H), 1.35 (d, *J* = 5.87 Hz, 3H), 0.97 (t, *J* = 7.34 Hz, 6H); ^{13}C NMR (101 MHz, MeOH-d_4) δ 167.7, 139.2, 131.6, 128.5, 127.5, 125.9, 125.0, 51.7, 50.2, 46.3, 35.4, 22.7, 21.2, 10.0; HRMS *m*/*z* calcd for $(C_{16}H_{24}N_2OSe + H)^+$ 341.1127, found 341.1133.

2,4-Difluoro-*N*-(2-(3-oxobenzo[*d*][1,2]selenazol-2(3*H*)-yl)ethyl)benzamide (11_a30)

Following the experimental procedure for the preparation of compound **11_a2** described above but with *N*-(2-aminoethyl)-2,4-difluorobenzamide (**a30**) (0.90 g, 4.5 mmol) as a starting material, compound **11_a30** (0.17 g) was obtained in 22% yield. ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.94 (t, *J* = 7.82 Hz, 2H), 7.80 (q, *J* = 7.83 Hz, 1H), 7.62 (t, *J* = 7.34 Hz, 1H), 7.38 - 7.53 (m, 1H), 7.05 (t, *J* = 8.80 Hz, 2H), 4.07 (t, *J* = 4.89 Hz, 2H), 3.73 (t, *J* = 5.38 Hz, 2H); ^{13}C NMR (101 MHz, MeOH-d_4) δ 168.3, 164.8 (d, *J* = 2.20 Hz), 164.6 (dd, *J* = 253.09, 12.47 Hz), 160.7 (dd, *J* = 253.09, 12.47 Hz), 140.0, 132.0 (dd, *J* = 10.27, 3.67 Hz), 131.7, 127.4, 127.3, 125.7, 124.9, 124.8, 119.2 (dd, *J* = 13.20, 3.67 Hz), 111.4 (dd, *J* = 22.01, 3.67 Hz), 104.0 (dd, *J* = 27.14, 25.68 Hz), 43.2, 39.7; HRMS *m/z* calcd for $(C_{16}H_{12}F_2N_2O_2Se + H)^+$ 383.0105, found 383.0100.

_N_-(2-(3-oxobenzo[_d_][1,2]selenazol-2(3_H_)-yl)ethyl)thiophene-2-carboxamide (11_a31)

Following the experimental procedure for the preparation of compound **11_a2** described above but with *N*-(2-aminoethyl)thiophene-2-carboxamide (**a31**) (0.76 g, 4.5 mmol) as a starting material, compound **11_a31** (56 mg) was obtained in 8% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.71 (br. s., 1H), 8.02 (d, *J* = 6.85 Hz, 1H), 7.84 (d, *J* = 6.85 Hz, 1H), 7.75 (br. s., 2H), 7.61 (br. s., 1H), 7.29 - 7.52 (m, 1H), 7.15 (br. s., 1H), 3.89 (m., 2H), 3.51 (m., 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.0, 161.9, 140.3, 140.2, 131.9, 131.2, 128.7, 128.3, 128.2, 127.8, 126.3, 126.2, 43.3. LRMS *m/z* calcd for $(C_{16}H_{12}F_2N_2O_2Se + H)^+$ 353.3, found 353.4.

2-(2-(Tetrahydro-2_H_-pyran-4-yl)ethyl)benzo[_d_][1,2]selenazol-3(2_H_)-one (11_a32)

Following the experimental procedure for the preparation of compound **11_a2** described above but with 2-(tetrahydro-2*H*-pyran-4-yl)ethanamine (**a32**) (0.58 g, 4.5 mmol) as a starting material, compound **11_a32** (0.40 g) was obtained in 64% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, *J*

= 7.83 Hz, 1H), 7.68 (d, *J* = 7.82 Hz, 1H), 7.56 (t, *J* = 7.34 Hz, 1H), 7.33 - 7.48 (m, 1H), 3.82 - 4.01 (m, 4H), 3.32 (t, *J* = 11.74 Hz, 2H), 1.61 - 1.71 (m, 4H), 1.53 - 1.61 (m, 1H), 1.25 - 1.38 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.2, 137.8, 131.9, 128.7, 127.6, 126.2, 124.2, 67.9, 42.0, 37.5, 32.8, 32.2; HRMS *m/z* calcd for $(C_{14}H_{17}NO_2Se + H)^+$ 313.0498, found 313.0512.

2-(Prop-2-yn-1-yl)benzo[*d*][1,2]selenazol-3(2*H*)-one (11_a33)

Following the experimental procedure for the preparation of compound **11_a2** described above but with prop-2-yn-1-amine (**a33**) (0.25 g, 4.5 mmol) as a starting material, compound **11_a33** (0.24 g) was obtained in 51% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, *J* = 7.82 Hz, 1H), 7.55 - 7.72 (m, 2H), 7.44 (t, *J* = 7.34 Hz, 1H), 4.67 (d, *J* = 1.96 Hz, 2H), 2.49 (t, *J* = 2.45 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.8, 138.1, 132.3, 128.9, 127.0, 126.3, 124.1, 74.4, 34.3; HRMS *m/z* calcd for $(C_{10}H_7NOSe + H)^+$ 238.9745, found 238.9768.

Dimethyl (2-(3-oxobenzo[*d*][1,2]selenazol-2(3*H*)-yl)ethyl)phosphoramidate (11_a34)

Following the experimental procedure for the preparation of compound **11_a2** described above but with dimethyl (2-aminoethyl)phosphoramidate (**a34**) (0.63 g, 4.5 mmol) as a starting material, compound **11_a34** (0.18 g) was obtained in 26% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, *J* = 7.83 Hz, 1H), 7.70 (d, *J* = 7.82 Hz, 1H), 7.54 - 7.62 (m, 1H), 7.40 (t, *J* = 7.82 Hz, 1 H), 3.94 (t, *J* = 5.87 Hz, 2H), 3.66 (s, 3H), 3.64 (s, 4H), 3.22 - 3.32 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.8, 138.5, 132.1, 128.6, 127.0, 126.2, 124.2, 77.4, 77.1, 76.8, 53.2, 53.1, 45.9, 45.9, 41.5, 28.4; HRMS *m/z* calcd for $(C_{11}H_{15}N_2O_4PSe + H)^+$ 351.0007, found 351.0012.

2-(2-Hydroxyethyl)benzo[*d*][1,2]selenazol-3(2*H*)-one (11_a35)

Following the experimental procedure for the preparation of compound **11_a2** described above but with 2-aminoethanol (**a35**) (0.27 g, 4.5 mmol) as a starting material, compound **11_a35** (0.24 g) was obtained in 50% yield. ^{1}H NMR (400MHz, DMSO-d_6) δ 8.05 (d, J = 7.8 Hz, 1 H), 7.83 (d, J = 7.8 Hz, 1 H), 7.60 (t, J = 7.3 Hz, 1 H), 7.40 (t, J = 7.3 Hz, 1 H), 5.11 (br. s., 1 H), 3.81 (t, J = 5.4 Hz, 2 H), 3.71 - 3.58 (m, 2 H); ^{13}C NMR (101MHz, MeOH-d_4) δ 168.3, 140.7, 131.6, 127.3, 127.2, 125.6, 124.7, 60.7, 46.6; HRMS *m/z* calcd for $(C_9H_9NO_2Se + H)^+$ 243.9877, found 243.9869.

2-(3-(1*H*-imidazol-1-yl)propyl)benzo[*d*][1,2]selenazol-3(2*H*)-one (11_a36)

Following the experimental procedure for the preparation of compound **11_a2** described above but with 1-(3-aminopropyl)imidazole (**a36**) (0.58 g, 4.5 mmol) as a starting material, compound **11_a36** (0.22 g) was obtained in 36% yield. ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.96 (d, J = 7.82 Hz, 2H), 7.84 (s, 1H), 7.65 (t, J = 7.34 Hz, 1H), 7.48 (t, J = 7.34 Hz, 1H), 7.26 (s, 1H), 7.03 (s, 1H), 4.15 (t, J = 7.34 Hz, 2H), 3.88 (t, J = 6.36 Hz, 2H), 2.26 (quin, J = 6.85 Hz, 2H); ^{13}C NMR (101 MHz, MeOH-d_4) δ 168.2, 139.5, 137.0, 131.8, 127.5, 127.2, 125.9, 125.0, 119.4, 119.3, 44.1, 41.2, 31.3; HRMS *m/z* calcd for $(C_{13}H_{13}N_3OSe + H)^+$ 308.0300, found 308.0302.

2-(3-Oxobenzo[*d*][1,2]selenazol-2(3*H*)-yl)acetic acid (11_a37)

A solution of compound **10** (0.51 g, 2 mmol) in DCM was added dropwise into the solution of glycine (**a37**) (0.34 g, 4.5 mmol) in acetonitrile. The mixture was stirred under room temperature for 48 hours. After the stirring, acetonitrile was removed under reduced pressure. The oily residue was dissolved in diethyl ether and stirred in diluted hydrochloric acid solution (1.5 mL HCl in 40 mL H_2O) overnight until the formation precipitate. The precipitate was filtered off,

washed with H_2O and recrystallized in MeOH/H_2O (3:2) to furnish the title compound **11_a37** (0.35 g) in 68% yield. ^{1}H NMR (400MHz, Acetone-d_6) δ 8.08 (d, *J* = 7.8 Hz, 1 H), 7.93 (d, *J* = 7.8 Hz, 1 H), 7.64 (t, *J* = 6.8 Hz, 1 H), 7.45 (t, *J* = 7.3 Hz, 1 H), 4.53 (s, 2 H); ^{13}C NMR (101MHz, Acetone-d_6) δ 170.1, 167.3, 140.3, 131.7, 127.7, 127.3, 125.7, 125.5, 44.8; HRMS *m*/*z* calcd for $(C_9H_7NO_3Se + H)^+$ 256.9591, found 257.9664.

***tert*-Butyl (2-(3-oxobenzo[*d*][1,2]selenazol-2(3*H*)-yl)ethyl)carbamate (11_a38)**

Following the experimental procedure for the preparation of compound **11_a2** described above but with *N*-Boc-ethylenediamine (**a38**) (0.72 g, 4.5 mmol) as a starting material, compound **11_a38** (0.52 g) was obtained in 76% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, *J* = 7.82 Hz, 1H), 7.55 - 7.72 (m, 2H), 7.44 (t, *J* = 7.34 Hz, 1H), 5.12 (br. s., 1H), 3.98 (t, *J* = 5.38 Hz, 2H), 3.40 - 3.54 (m, 2H), 1.44 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.2, 132.1, 128.8, 126.9, 126.3, 124.0, 79.6, 44.5, 40.9, 28.4; HRMS *m*/*z* calcd for $(C_{14}H_{18}N_2O_3Se + Na)^+$ 366.0370, found 366.0372.

***tert*-Butyl (3-(3-oxobenzo[*d*][1,2]selenazol-2(3*H*)-yl)propyl)carbamate (11_a39)**

Following the experimental procedure for the preparation of compound **11_a2** described above but with *N*-Boc-1,3-propanediamine (**a39**) (0.78 g, 4.5 mmol) as a starting material, compound **11_a39** (0.32 g) was obtained in 46% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, *J* = 7.83 Hz, 1H), 7.67 (d, *J* = 7.83 Hz, 1H), 7.52 (t, *J* = 6.85 Hz, 1H), 7.31 - 7.40 (m, 1H), 5.56 (br. s., 1H), 3.87 (t, *J* = 6.36 Hz, 2H), 3.03 - 3.14 (m, 2H), 1.80 (quin, *J* = 6.36 Hz, 2H), 1.36 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.7, 156.1, 138.1, 132.0, 128.7, 127.1, 126.2, 124.3, 79.0, 77.5,

77.2, 76.9, 41.9, 36.9, 30.4, 28.4; HRMS *m/z* calcd for $(C_{15}H_{20}N_2O_3Se + H)^+$ 357.0713, found 357.0726.

3-Butyl-1-(3-(3-oxobenzo[*d*][1,2]selenazol-2(3*H*)-yl)propyl)-1*H*-imidazol-3-ium iodide (11_a40)

Reaction of compound **11_a36** (0.30 g, 1 mmol) with 1-butyl iodide (0.22 g, 1.2 mmol) using DMF (5 mL) as the solvent at room temperature gave the title compound **11_a40** (0.04 g) in 12% yield. ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.77 (d, *J* = 1.71 Hz, 1H), 7.69 - 7.74 (m, 1H), 7.59 (d, *J* = 7.82 Hz, 1H), 7.50 (dd, *J* = 1.47, 7.58 Hz, 1H), 7.41 (dt, *J* = 1.34, 7.64 Hz, 1H), 7.26 - 7.36 (m, 1H), 4.42 (t, *J* = 6.72 Hz, 2H), 4.22 - 4.34 (m, 2H), 3.44 (t, *J* = 6.36 Hz, 2H), 3.04 - 3.13 (m, 2H), 1.85 - 2.01 (m, 2H), 1.68 - 1.76 (m, 2H), 1.28 - 1.37 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.8, 141.7, 135.0, 134.7, 134.4, 131.3, 129.7, 126.5, 126.4, 61.5, 57.1, 53.4, 46.2, 46.2, 39.7, 35.6, 35.5, 33.7, 30.2, 26.6, 23.3, 23.1, 16.5, 16.5, 16.4, 14.2; HRMS *m/z* calcd for $(C_{17}H_{22}N_3OSe + H)^+$ 364.0928, found 364.0925.

2-(2-Aminoethyl)benzo[*d*][1,2]selenazol-3(2*H*)-one hydrochloride (11_a41)

Hydrochloric acid (3 mL) was added dropwise into a solution of compound **11_a38** (0.50 g, 1.5 mmol) in MeOH (5 mL) and DCM (5 mL) at 0 °C. The mixture was stirred for 14 h. The solvent was evaporated under reduced pressure. MeOH and ether were added to form precipitates, which were filtered and washed twice with ether to furnish the title compound **11_a41** (0.25 g) in 61% yield. ^{1}H NMR (400 MHz, D_2O) δ 7.76 (d, *J* = 7.82 Hz, 2H), 7.57 (t, *J* = 7.34 Hz, 1H), 7.36 - 7.43 (m, 1H), 3.98 - 4.05 (m, 2H), 3.23 - 3.30 (m, 2H); ^{13}C NMR (101 MHz, D_2O) δ 169.8,

139.7, 132.8, 127.7, 126.6, 126.2, 124.9, 42.1, 39.6; HRMS *m/z* calcd for $(C_9H_{10}N_2OSe + H)^+$ 243.0037, found 243.0036.

2-(3-Aminopropyl)benzo[*d*][1,2]selenazol-3(2*H*)-one hydrochloride (11_a42)

Hydrochloric acid (3 mL) was added dropwise into a mixture of compound **11_a39** (0.50 g, 1.4 mmol) in MeOH (5 mL) and DCM (5 mL) at 0 °C. The mixture was stirred for 14 h. The solvent was evaporated under reduced pressure. MeOH and ether were added to form precipitates, which were filtered and washed twice with ether to furnish the title compound **11_a42** (0.21 g) in 51% yield. ^{1}H NMR (400 MHz, D_2O) δ 7.55 (d, *J* = 7.82 Hz, 1H), 7.48 (d, *J* = 7.82 Hz, 1H), 7.33 (t, *J* = 7.34 Hz, 1H), 7.15 (t, *J* = 7.34 Hz, 1H), 3.63 (t, *J* = 6.85 Hz, 2H), 2.84 (t, *J* = 7.34 Hz, 2H), 1.82 - 1.94 (m, 2H); ^{13}C NMR (101 MHz, D_2O) δ 168.5, 139.3, 132.3, 127.3, 126.3, 126.1, 124.7, 41.4, 36.7, 27.4; HRMS *m/z* calcd for $(C_{10}H_{12}N_2OSe + H)^+$ 257.0184, found 257.0193.

1,1,1-Trifluoro-2-methylpropan-2-yl (2-(3-oxobenzo[*d*][1,2]selenazol-2(3*H*)-yl)ethyl)carbamate (11_a43)

Further treatment of compound **11_a41** (0.28 g, 1.0 mmol) with 3-methyl-1-(((1,1,1-trifluoro-2-methylpropan-2-yl)oxy)carbonyl)-1*H*-imidazol-3-ium iodide (0.44 g, 1.2 mmol) in the NEt_3 (5 mL) using $CHCl_3$ (5 mL) as the solvent produced the title compound **11_a43** (0.36 g) in 46% yield. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.01 (d, *J* = 7.83 Hz, 1H), 7.93 (d, *J* = 7.82 Hz, 1H), 7.59 - 7.71 (m, 1H), 7.42 - 7.52 (m, 1H), 6.79 (br. s., 1H), 3.94 (t, *J* = 5.87 Hz, 2H), 3.45 (q, *J* = 5.71 Hz, 2H), 1.65 (s, 6H); ^{13}C NMR (101 MHz, Acetone-d_6) δ 131.7, 127.9, 127.6, 125.8, 125.1, 43.5, 40.7, 29.5, 29.3, 29.1, 28.9, 28.7, 28.6, 28.4, 19.0; ^{19}F NMR (376 MHz, Acetone-d_6) δ −85.23; HRMS *m/z* calcd for $(C_{14}H_{15}F_3N_2O_3Se + H)^+$ 397.0273, found 397.0278.

1,1,1-Trifluoro-2-methylpropan-2-yl (3-(3-oxobenzo[*d*][1,2]selenazol-2(3*H*)-yl)propyl)carbamate (11_a44)

Further treatment of compound **11_a42** (0.29 g, 1.0 mmol) with 3-methyl-1-(((1,1,1-trifluoro-2-methylpropan-2-yl)oxy)carbonyl)-1*H*-imidazol-3-ium iodide (0.44 g, 1.2 mmol) in the NEt_3 (5 mL) using $CHCl_3$ (5 mL) as the solvent generated the title compound **11_a44** (0.38 g) in 47% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, *J* = 7.82 Hz, 1H), 7.57 - 7.71 (m, 2H), 7.46 (t, *J* = 6.85 Hz, 1H), 5.75 (br. s., 1H), 3.95 (t, *J* = 5.87 Hz, 2H), 3.11 - 3.25 (m, 2H), 1.82 - 1.95 (m, 2H), 1.69 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.1, 132.2, 128.9, 126.9, 126.4, 124.1, 79.2, 77.3, 77.0, 76.7, 50.0, 41.8, 37.1, 30.2, 29.7; ^{19}F NMR (376 MHz, Acetone-d_6) δ -84.67, -84.75, -84.98; HRMS *m/z* calcd for $(C_{15}H_{17}F_3N_2O_3Se + H)^+$ 411.0430, found 411.0433.

Benzyl (2-(3-oxobenzo[*d*][1,2]selenazol-2(3*H*)-yl)ethyl)carbamate (11_a45)

Benzyl chloroformate (0.10 mL) was added dropwise into the mixture of NEt_3 (0.10 mL) and compound **11_a41** (0.10 g, 0.41 mmol) in DCM (10 mL) at 0 °C. The mixture was stirred for 14 h. Then the solvent was removed and the residue was dissolved in DCM, and the organic layer was washed twice with H_2O and brine once. The organic layers were combined and dried over anhydrous $MgSO_4$ and concentrated in vacuum. The crude product was purified by column chromatography on silica gel to give the title compound **11_a45** (26 mg) in 16% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, *J* = 7.82 Hz, 1H), 7.59 - 7.67 (m, 2H), 7.41 - 7.49 (m, 1H), 7.29 - 7.41 (m, 5H), 5.39 (br. s., 1H), 5.13 (s, 2H), 3.93 - 4.06 (m, 2H), 3.52 - 3.61 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.8, 156.5, 138.1, 136.5, 132.2, 128.9, 128.5, 128.1, 126.8, 126.3, 124.0,

77.3, 77.2, 77.0, 76.7, 66.8, 44.4, 41.4; HRMS *m/z* calcd for $(C_{17}H_{16}N_2O_3Se + Na)^+$ 399.0219, found 399.0220.

Benzo[*f*][1,2,4]selenadiazepine-3,5(*2H,4H*)-dione (12)

A solution of compound **10** (0.51 g, 2 mmol) in DCM was added dropwise into a stirred suspension of urea in acetonitrile cooled in an ice salt bath. After stirring for 24 h, the solvent was evaporated under reduced pressure. The residue was washed twice with water and the suspension was neutralized with Na_2CO_3 solution. The crude product was filtered off, washed twice with water, and recrystallized in acetonitril/benzene (4:1) to furnish the title compound **12** (0.14 g) in 30% yield. ^{1}H NMR (400MHz, DMSO-d_6) δ 8.28 (br. s., 1 H), 8.07 (d, *J* = 7.8 Hz, 1 H), 7.97 (br. s., 1 H), 7.91 (d, *J* = 7.8 Hz, 1 H), 7.73 (t, *J* = 7.3 Hz, 1 H), 7.48 (t, *J* = 7.3 Hz, 1 H); ^{13}C NMR (101MHz, DMSO-d_6) δ 166.3, 153.8, 139.6, 134.0, 129.5, 128.7, 126.7, 126.5; HRMS *m*/*z* calcd for $(C_8H_6N_2O_2Se + H)^+$ 241.9594, found 242.2845.

2-Phenyl-1,2-selenazolidin-3-one (13_a1)

To a suspension of 3,3'-diselanediyldipropanoic acid (**9b**) (0.61g, 2 mmol) in DCM cooled in an ice bath, EDCI (0.37 g, 2.4 mmol.) was added in portions. After stirring for two hours, the reaction mixture became clear and aniline (0.56 g, 6 mmol) was added dropwise into the solution. The mixture was stirred at room temperature for another 14 h. After the reaction was completed, the precipitate was filtered, washed twice with water and acetone to yield 3,3'-diselanediylbis(*N*-phenylpropanamide) (0.73 g) in 80% yield, which was pure enough for use in the next step. To a solution of 3,3'-diselanediylbis(*N*-phenylpropanamide) (0.8 g, 1.5 mmol) in MeOH at room temperature, *t*-BuOOH (0.40 g, 4.5 mmol) dissolved in MeOH was added

dropwise. The mixture was stirred at room temperature for 24 h. After the reaction was completed, the mixture was diluted with MeOH, filtered, and the filtrate was collected and concentrated under reduced pressure. The obtained residue was purified by column chromatography on silica gel with EA/Hex (1:4) as eluent to furnish the title compound **13_a1** (15 mg) in 5% yield. ^{1}H NMR (400MHz, MeOH-d_4) δ 7.55 (d, J = 7.8 Hz, 2 H), 7.30 (t, J = 7.8 Hz, 2 H), 7.14 - 7.06 (m, 1 H), 3.26 (t, J = 7.3 Hz, 2 H), 2.91 (t, J = 7.3 Hz, 2 H); ^{13}C NMR (101MHz, MeOH-d_4) δ 132.3, 122.3, 117.8, 113.8, 31.6, 17.9; HRMS m/z calcd for (C_9H_9NOSe + H)$^+$ 227.9922, found 227.9927.

Materials for biological studies

Meropenem, and ebselen were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Luria broth (LB) and nitrocefin were purchased from BD (Franklin Lakes, NJ, USA). Mueller-Hinton broth (MHB) was purchased from Oxoid Co. (Hampshire, United Kingdom). Isopropyl β-D-1-thiogalactopyranoside (IPTG) was purchased from IBI Inc. (Boca Raton, FL, USA). The $bla_{\text{NDM-1}}$ gene was PCR amplified and constructed in an IPTG-inducible pET28b vector.[32] The constructed plasmid was transformed with *E. coli* BL21 to form a strain of *E. coli* BL21 (NDM-1) harboring the recombinant plasmid pET28b-$bla_{H6\text{-}NDM\text{-}1}$, which encoded G^{36} to R^{270} and carried an N-terminal His_6 tag for the overexpression and purification of the NDM-1 enzyme. *E. coli* TG1 was transformed with the IncX3 $bla_{NDM\text{-}1}$-bearing plasmid (similar as plasmid, pP855-NDM5, MF547508.1) originally isolated from a clinical *K. Pneumoniae* and was used in the preliminary MIC screening of test compounds alone and in combination with Meropenem. Clinically isolated CRE strains shown in **Table 2** were from our in-house bacterial strain library,

which were isolated from different specimens (urine, faeces, and sputum) collected from patients in hospitals in Zhejiang Province, China.[33]

Antimicrobial susceptibility tests and FIC index determination

The MIC values of all compounds and their combination with Mem were determined and interpreted in accordance with the CLSI guidelines[27] and previous report.[24] At least three independent assays were performed for each compound and their combination with Mem. FIC index was calculated as FIC (compound) + FIC (Mem), where FIC (compound) is the (MIC of compound in combination with Mem) / (MIC of compound alone) while FIC (Mem) is (MIC of compound in combination with Mem) / (MIC of Mem alone). FIC index of ≤0.5 was deemed synergistic.

Cytotoxicity tests towards normal cells

The standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cytotoxicity assay was performed.[34] A solution of compound **11_a38** in DMSO at a concentration of 400 mg/mL was freshly prepared as a stock solution. Eukaryotic cells (L929 or Hela) were seeded into three 96-well plates at a density of 1×10^4 cells per well in DMEM (10% FBS) and incubated for 12 h at 37 °C. The cells were then exposed to various concentrations of compound **11_a38** (0.13 mg/mL to 2 mg/mL) for 48 h. Medium containing 0.5% DMSO and medium without cells were used as negative control and blank control respectively. After incubation, MTT at a concentration of 0.5 mg/mL in PBS was added to each well and the cells were further incubated for 3 h at 37 °C. The medium was removed to afford the formazan crystals followed by dissolving in DMSO. The optical density (OD) of each well was measured at 490 nm using a

Microplate Reader (Clariostar, BMG). The percentage of survival cell was calculated using the formula: (corrected reading from test well − corrected reading from blank well)/ (corrected reading from negative well − corrected reading from blank well) × 100%.

Evaluation of the *in vivo* synergistic activity using a *G. mellonella* model of infection

To evaluate the *in vivo* synergistic efficacy of compound **11_a38** in combination with Mem, an infection model of *G. mellonella* was employed as previously described with little modification.[35] In brief, 1 mL aliquots of overnight cultures of clinical CRE isolate *E. cloacae* EL10 were pelleted and washed twice with sterile phosphate buffered saline (PBS) before being resuspended in 100 μL of PBS. *G. mellonella* larvae (N = 10) at weight 250 – 300 mg were selected for inoculation with a lethal dose of 10 μL bacterial suspensions (2.5 x 10^5 CFU/larva). Using a 50 μL Hamiton syringe, the bacterial suspension was injected into the hemocoels at the last left proleg of larvae. Larvae were then treated with various treatments at 1 h before bacterial inoculation. Treatments included vehicle, compound **11_a38** along, Mem along, compound **11_a38** in combination with Mem. Treatments were performed in the same manner as infection, except that injections were into the next left proleg moving toward the head of the larvae. Larvae were then incubated in Petri dishes at 37 °C and mortality rates were monitored at 12 h interval for 48 h. Larvae were considered dead if they did not respond to physical stimuli. Data were analyzed for statistical significance using a log-rank and χ square test with 1 degree of freedom.

Overexpression, purification and kinetic assay of NDM-1 protein

The purified NDM-1 protein was prepared as previously described.[32] Kinetic assay of NDM-1 was performed to determine the inactivation constants of compounds as previously described.[24]

Briefly, followed by addition of 7 fold of Km of the reporter substrate nitrocefin, 1 nM of pure NDM-1 was mixed with different concentrations of compounds in 500 µL of 50 mM phosphate buffer with or without 50 µM $ZnSO_4$. Bovine serum albumin (BSA) was then added to stabilize the activity of diluted NDM-1. The readout of the velocity can be recorded by the wavelength change at 482 nm. Independent assay was performed in triplicate.

ESI-MS analysis of NDM-1 with compound 11_a38

Waters Synapt G2-Si electrospray ionization/quadrupole-ion mobility-time-of flight mass spectrometer was employed to perform the Electrospray ionization mass spectrometry (ESI-MS) experiments. For qualitative detection of the binding between NDM-1 and compound under non-denaturing conditions, after incubating 20 µM of NDM-1 in 20 mM ammonia acetate with equal molar of compound in the same buffer system for 20 mins, the reaction mixture was infused directly into a nanospray emitter (Econo12, New Objectives, Woburn, USA), which was mounted onto a nano-ESI source for analysis. The spray voltage was carefully raised to initiate the spray process, which was maintained for around 20 mins at the voltage of 150V.[36] For analysis under denaturing conditions, an equal volume of acetonitrile with 0.5% formic acid (v/v) was added to the NDM-1/ligand reaction mixture before being loaded to the ESI source with a syringe pump at a flow rate of 5 µL/min. During data acquisition, positive ion mode was exhibited in the operation of the mass spectrometer in the *m/z* range of 200-5000 for detection of multiply charged ions. The Transform program (MassLynx 4.1, Waters) was used to analyze the obtained raw multiply charged mass spectra.

ASSOCIATED CONTENT

Supporting Information. ^{1}H NMR and ^{13}C NMR of compounds **9a**, **10** – **13**, **Figure S49**-**S51**, and HPLC chromatogram of compound **11_a38**.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

We acknowledge the support by the Research Grants Council of Hong Kong (grant no 15100115 and 25100014), the Collaborative Research Fund from the Research Grant Council of the Government of Hong Kong SAR (C5026-16G), the Innovation and Technology Commission,

and The Hong Kong Polytechnic University. We also thank Mr. Xuezhen Zhu for assisting the cytotoxicity studies of compound **11_a38**.

ABBREVIATIONS

CRE, carbapenem-resistant *Enterobacteriaceae*; CDC, Centers for Diseases Control and Prevention; WHO, World Health Organization; MBLs, metallo-β-lactamases; NDM-1, New Delhi metallo-β-lactamase-1; Eb, ebselen; IPTG, isopropyl-β-D-thiogalactoside; Mem, meropenem; MIC, minimum inhibition concentration; FIC, fractional inhibitory concentration; SAR, structure-activity relationship; ESI-MS, electrospray ionization mass spectrometry; DMF, dimethylformamide; EDCI, *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide; DCM, dichloromethane; CLSI, Clinical and Laboratory Standards Institute.

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Highlights

- A focused compound library of forty-six 1,2-benzisoselenazol-3(2*H*)-one derivatives was designed and synthesized.
- Most of these compounds exhibited strong synergistic activity when combined with meropenem against NDM-1-producing carbapenem-resistant *Enterobacteriaceae* isolates.
- MS analysis indicated that compound could covalently bind to the purified NDM-1 enzyme and displaced one zinc ion from the active site, therefore inhibiting the NDM-1 activity.