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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Rational exploration of new pyridinium-based HSP90α inhibitors tailored to thiamine structure

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Abstract The anticancer activity of thiamine (vitamin B1) combined with its structural properties and docking studies suggested potential anti-heat shock protein 90α (Hsp90α) activity for this vitamin. In experimental testing, thiamine illustrated anti-Hsp90α IC_{50} value of 12.5 μM. Therefore, in an attempt to capitalize on the simple structure of thiamine and towards the development of new anti-Hsp90α inhibitors, we prepared and screened 56 pyridinium-based structures tailored to thiamine. The most potent among the prepared compounds illustrated anti-Hsp90α IC_{50} values of 7.4 and 7.6 μM.

Keywords Thiamine · Heat shock protein · Hsp90α · Cancer · Docking simulations · Pyridinium

Introduction

Hsp90α is a molecular chaperone that plays crucial role in the conformational maturation, stability, and function of protein substrates within the cell (Prodromou and Pearl, 2003). The interaction of ATP with its binding domain in Hsp90α leads to autophosphorylation of certain tyrosine residues, thus activating this kinase and provides the necessary energy for refolding of denatured proteins (Prodromou and Pearl, 2003).

Amongst the client proteins of Hsp90α are many oncogenes essential for the survival, proliferation, invasion, metastasis, and angiogenesis of tumors. In fact, 48 oncogenic proteins have been shown to be dependent upon Hsp90α for conformational activation, including: telomerase, Her2 (erbB2), Raf-1, focal adhesion kinase, and the steroid hormone receptors (Christopher *et al.* 1991).

The validity of Hsp90α as anticancer target for drug discovery was established by emerging clinical trials employing the potent Hsp90α inhibitors 17-allylaminogeldanamycin, geldanamycin, and radicicol (Solit and Rosen, 2006; Chiosis *et al.*, 2006; Chiosis *et al.*, 2002; Neckers *et al.*, 2009; Xiao *et al.*, 2006).

However, despite the high cellular activity and clinical progression of 17-allylaminogeldanamycin (Hideyuki *et al.*, 2008), it has several limitations, e.g., poor solubility, hepatotoxicity, and extensive metabolism. These issues have led to significant efforts to identify novel rationally designed small molecular inhibitors of Hsp90α (Kasibhatla *et al.*, 2007).

Although thiamine (vitamin B1) was observed to have a high stimulatory effect on tumor growth at doses up to 25 times the recommended dietary allowance (RDA), at very high overdoses of thiamine (i.e., >2500 times the RDA) it has been found to cause 10% inhibition of tumor growth. This effect was heightened, resulting in a 36% decrease, when thiamine supplementation was administered prior to tumor inoculation. The tumor inhibitory effect at high doses of thiamine was unexplained (ComõÂn-Anduix *et al.*, 2001). This observation combined with the fact that thiamine can be successfully docked into ATP binding pocket of Hsp90α in a low energy conformation/pose (see Experimental), as in Fig. 1, prompted us to expect certain inhibitory potential for thiamine against Hsp90α. Subsequent experimental assessment validated this assumption

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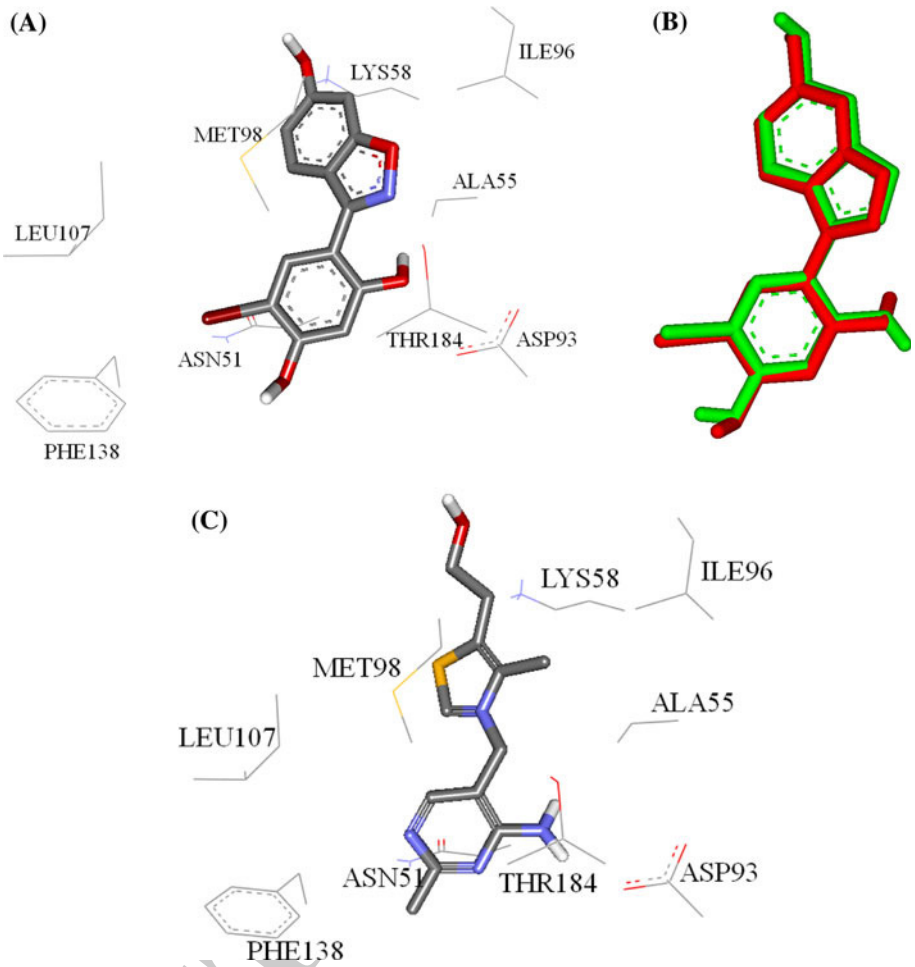

Fig. 1 **a** The benzoisoxazole derivative reported by Gopalsamy *et al.* (BXZ1) cocrystalized into Hsp90α (PDB code 3BM9, resolution 1.6 Å), **b** Comparison between the docked conformer/pose of inhibitor BXZ1 (*green*) as produced by LigandFit docking simulation and the crystallographic structure of this inhibitor within Hsp90α (*red*), and **c** thiamine docked into the same protein using the same LigandFit docking paramters

and showed anti-Hsp90α IC_{50} value of 12.5 μM for thiamine.

Accordingly, we initiated an exploratory effort to evaluate a series of pyridinium-based compounds exemplified by compound **24** in Fig. 2. As shown in this figure, the two compounds, i.e., thiamine and **24**, share three hydrogen-bonding regions, a central aromatic system and a terminal hydrophobe. Furthermore, the central cationic center of the proposed pyridinium compounds equates the thiazolinium ring of thiamine, which might favorably contribute to binding by electrostatic attraction with ASP93 in Hsp90.

Results and discussion

Chemistry

Table 1 shows the prepared target compounds **22–77**, while Schemes 1, 2, 3, and 4 show the synthetic steps implemented towards their preparation. The synthesis commenced by preparing the chloroacetylated derivatives **1–14** offered by the reaction of the corresponding aryl amines or aryl alkyl amines with mono-chloroacetyl chloride (Scheme 1). The resulting mono-chloroacetamides were fused neat with pyridine or *N*-substituted nicotinamide derivatives at 150°C to yield the final products **22–74** (Scheme 3). Simpler arylalkyl pyridinium derivatives **75–77** were also prepared by neat fusion of benzyl, phenylethyl, or ethyloxycarbonyl methyl chloride with nicotinamide (Scheme 4).

Several *N*-substituted nicotinamide starting materials **15–21** were prepared via reaction of nicotinic acid with oxalyl chloride to form nicotinyl chloride followed by coupling with the particular amines, as in Scheme 2.

Anti-Hsp90α bioactivities and structure–activity relationships

The prepared compounds **22–77** were bioassayed against recombinant human Hsp90α (BioQuote, UK) employing malachite green-based detection of inorganic phosphate released by the ATPase action of Hsp90α (Lanzetta *et al.*, 1979; Rowlands *et al.*, 2004; Avila *et al.*, 2006a, b).

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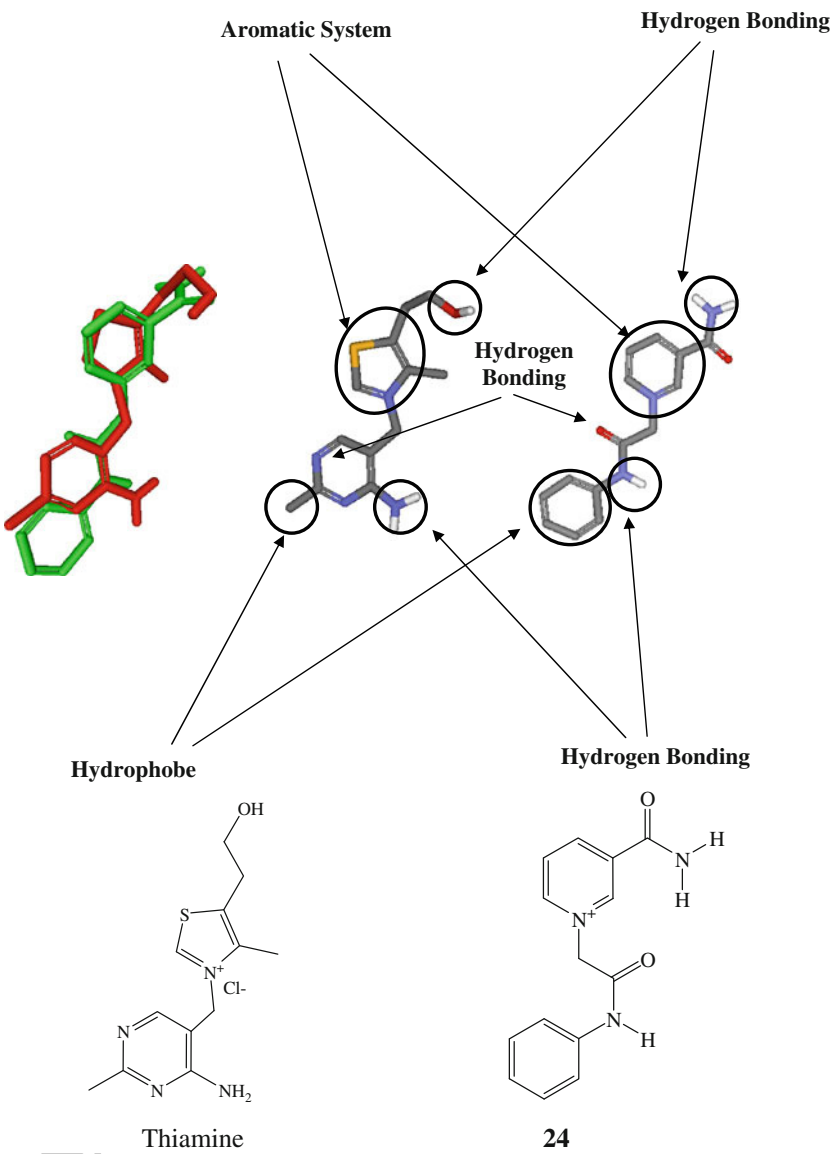

Fig. 2 The pharmacophoric similarities between thiamine (*red*) and the proposed pyridinium derivatives exemplified by **24** (*green*)

Geldanamycin was used as positive control to standardize our experimental setup (Dey and Cederbaum, 2007).

Several diverse substituents (i.e., electronically and hydrophobically) were explored on the acetamide fragment of the molecules (part A, Fig. 3), namely, *p*-nitrophenyl (as in **27**, **39**, **46**, **55**, **58**, **66** and **72**, Table 1), *p*-tolyl (as in **30**, **43**, **49**, **52**, **56**, **59**, **63**, **67** and **73**, Table 1), *p*-chlorophenyl (as in **25**, **37**, **47**, **51**, **60**, **68** and **74**, Table 1), and *p*-anisidyl (as in **26**, **38**, **48**, **54**, **57**, **62**, **65** and **71**, Table 1) groups, in addition to sulfonamidophenyl substitutions (as in **31**–**33**, **41**, **42**, **44**, **45**, **61**, **64** and **69**, Table 1). Furthermore, we explored the significance of *meta*-acetamido substituents at the pyrdinium ring (part B, Fig. 3) by preparing compounds with simple and extended *meta*-acetamido-side chains (nicotinamide derivatives: **22**–**34** and **45**–**74**, respectively), as well as, unsubstituted pyridinium derivatives **35**–**44**. Moreover, we explored the significance of the aniline-acetamido fragment in part A (Fig. 3) by evaluating the anti-Hsp90α potential of simpler compounds (i.e., **75**–**77**, Table 1).

Table 1 shows the chemical structures of the synthesized compounds and their anti-Hsp90α bioactivities. Apparently, the presence of unsubstituted *meta*-acetamido group on the pyridinium moiety (i.e., nicotinamide in part B, Fig. 3) enhances bioactivity (i.e., compared to the unsubstituted pyridinium derivatives) provided that the aryl-acetamido of part A (Fig. 3) is substituted with small fragments (H–, *p*-methyl and *m*-methyl, e.g., **24** vs. **36**; **30** vs. **43**; and **28** vs. **40**, respectively) and/or electron-withdrawing hydrophilic (–NO_2 and SO_2NH_2, e.g., **27** vs. **39** and **31** vs. **41**, respectively) or hydrophobic groups (–Cl, e.g., **25** vs. **37**). Otherwise, the *meta*-acetamido group in part B leads to lesser bioacitivites if combined with larger (benzyl, e.g., **22** vs. **35**) or electron-donating aryl groups

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Table 1 Chemical structure of synthetic compounds with Hsp90α inhibitory measurement

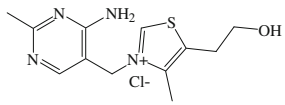
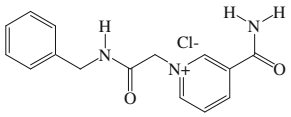
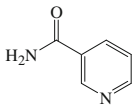
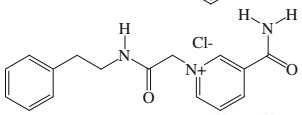
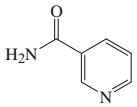
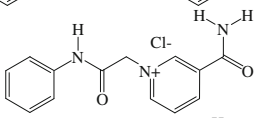
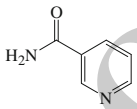
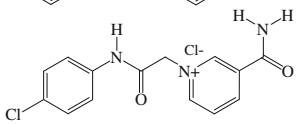
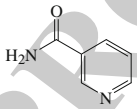
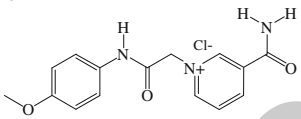
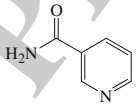
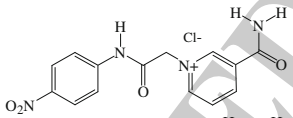
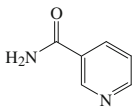
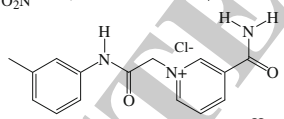
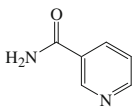
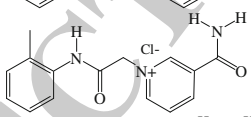
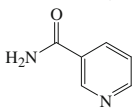
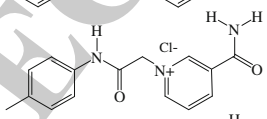
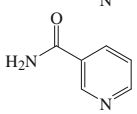
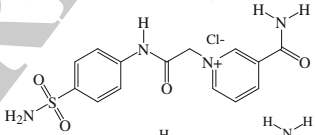
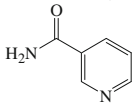
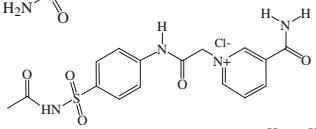
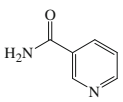
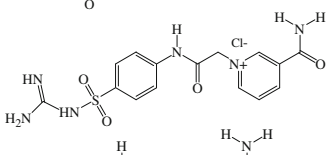
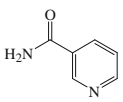
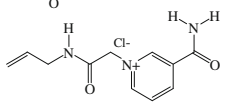
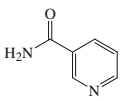
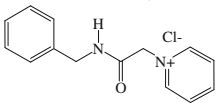
Compound	Chemical Structure	Reactants	% Inhibition at 10 μM	IC_{50} (μM)[e]
Thiamine[a]		--- ---	40%	12.5 (0.95)[b]
22		**1** 	20%	ND[c]
23		**2** 	46%	16.4 (0.99)
24		**3** 	37%	13.3 (0.95)
25		**4** 	24%	ND
26[d]		**5** 	20%	ND
27[d]		**6** 	62%	7.6 (0.97)
28[d]		**7** 	34%	13.2 (0.93)
29		**8** 	8%	ND
30[d]		**9** 	34%	42.2 (0.99)
31[d]		**10** 	32%	12.0 (0.89)
32[d]		**11** 	36%	12.7 (0.94)
33[d]		**12** 	29%	ND
34		**14** 	Inactive	ND
35		**1** 	36%	49.6 (0.99)

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Table 1 continued

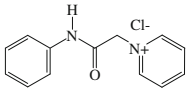
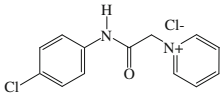
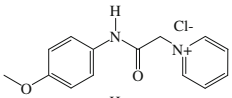
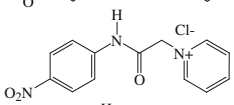
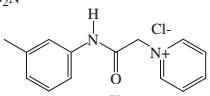
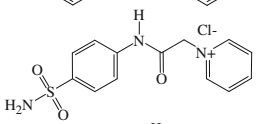
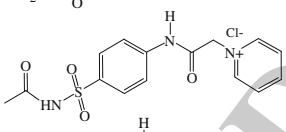
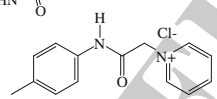
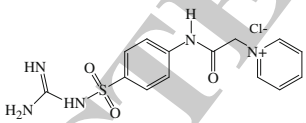
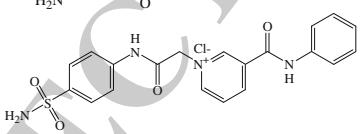
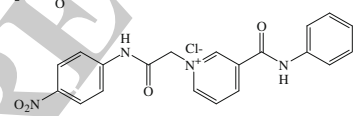
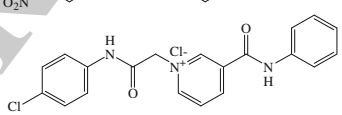
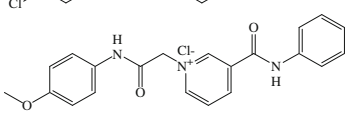
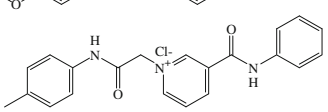
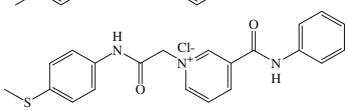
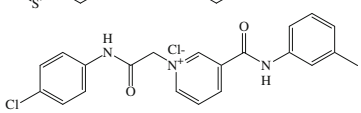
Compound	Chemical Structure	Reactants	%Inhibition at 10 μM	IC_{50} (μM)
36		**3** 	3%	ND
37		**4** 	3%	ND
38		**5** 	43%	13.3 (0.99)
39		**6** 	34%	14.9 (0.95)
40[d]		**7** 	12%	ND
41		**10** 	7%	ND
42[d]		**11** 	46%	12.7 (0.99)
43		**9** 	Inactive	ND
44[d]		**12** 	20%	ND
45[d]		**10** **15**	47%	26.6 (0.93)
46[d]		**6** **15**	11%	ND
47[d]		**4** **15**	17%	ND
48[d]		**5** **15**	4%	ND
49[d]		**9** **15**	59%	7.4 (0.99)
50[d]		**13** **15**	14%	ND
51[d]		**4** **16**	24%	ND

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Table 1 continued

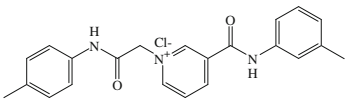
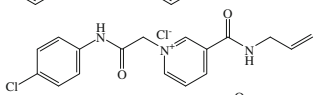
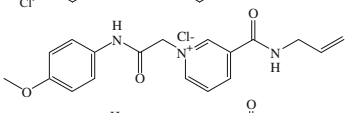
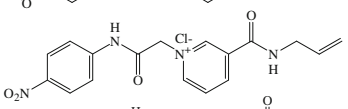
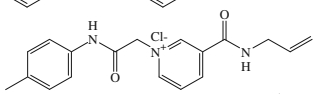
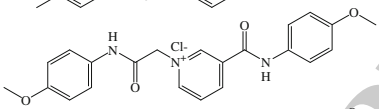
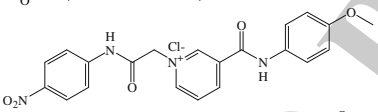
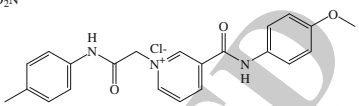
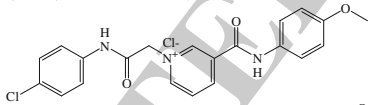
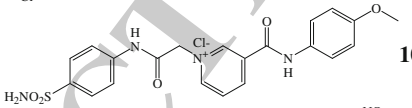
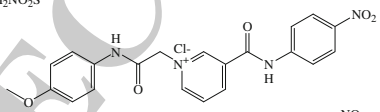
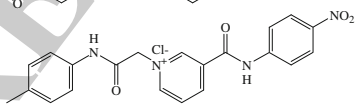
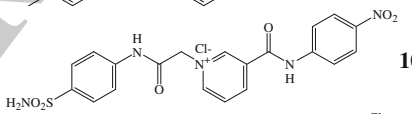
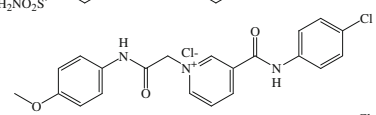
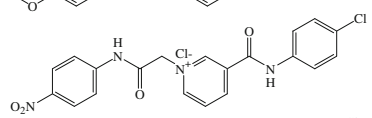
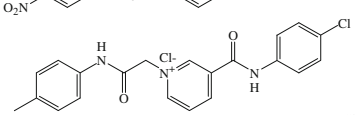
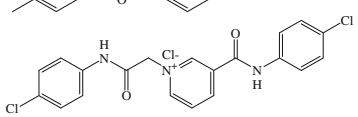
Compound	Chemical Structure	Reactants		%Inhibition at 10 μM	IC_{50} (μM)
52[d]		**9**	**16**	16%	ND
53		**4**	**21**	1.6%	ND
54[d]		**5**	**21**	32%	48.5 (0.97)
55[d]		**6**	**21**	20%	ND
56[d]		**9**	**21**	23%	ND
57[d]		**5**	**18**	3%	ND
58[d]		**6**	**18**	Inactive	ND
59[d]		**9**	**18**	20%	ND
60[d]		**4**	**18**	10%	ND
61[d]		**10**	**18**	23%	ND
62[d]		**5**	**19**	Inactive	ND
63[d]		**9**	**19**	21%	ND
64[d]		**10**	**19**	8%	ND
65[d]		**5**	**20**	2 %	ND
66[d]		**6**	**20**	24%	ND
67[d]		**9**	**20**	48%	17.3 (0.98)
68		**4**	**20**	Inactive	ND

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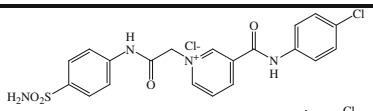
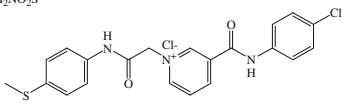
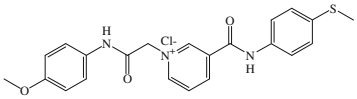
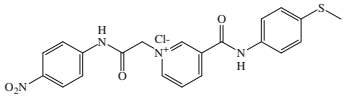
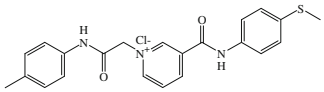
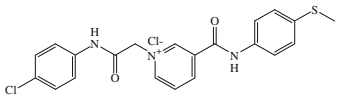
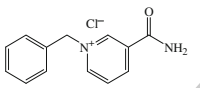
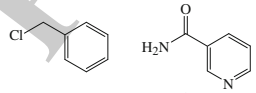
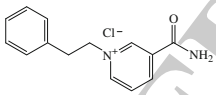
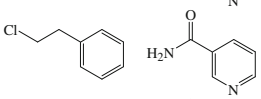
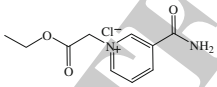
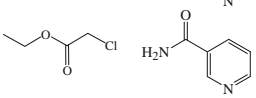
Table 1 continued

Compound	Chemical Structure	Reactants		%Inhibition at 10 μM	IC_{50} (μM)
69[d]		**10**	**20**	3%	ND
70		**13**	**20**	24%	ND
71[d]		**5**	**17**	29%	ND
72[d]		**6**	**17**	3%	ND
73[d]		**9**	**17**	18%	ND
74[d]		**4**	**17**	19%	ND
75				10%	ND
76				9%	ND
77[d]				13%	ND

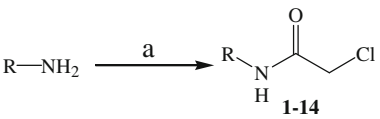
[a] Thaimine purity >98% (Sigma-Aldrich)

[b] Regression of three log cycle concentrations

[c] Not Determined

[d] Novel Compounds

[e] Geldanamycin was tested as reference standard and achieved IC_{50} value of 272 nM (0.98)



1-14

1 R = Benzyl

2 R = Phenylethyl

3 R = Phenyl

4 R = *p*-Chlorophenyl

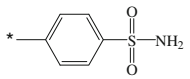
5 R = *p*-Methoxyphenyl

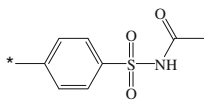
6 R = *p*-Nitrophenyl

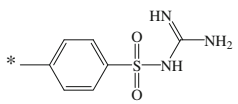
7 R = *m*-Tolyl

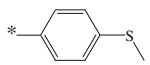
8 R = o-Tolyl

9 R = *p*-Tolyl

10 R = 

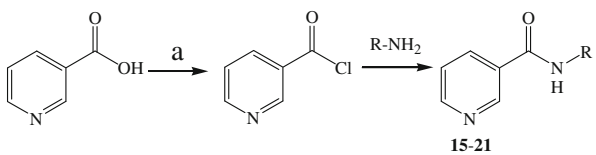
11 R = 

12 R = 

13 R = 

14 R = Allyl

Scheme 1 Synthesis of the mono-chloromethyl-acetamido- derivatives (**1**–**14**); (*a*) mono chloroacetylchloride/triethylamine in dry acetone



15-21

15 R = Phenyl

16 R = *m*-Tolyl

17 R = *p*-methylthiophenyl

18 R = *p*-Methoxyphenyl

19 R = *p*-Nitrophenyl

20 R = *p*-Chlorophenyl

21 R = Allyl

Scheme 2 Synthesis of nicotinic acid derivatives **15**–**21**. (*a*) Oxalyl chloride

at part A (MeO, e.g., **26** vs. **38**), as in Table 1. We believe this behavior is related to different binding modes assumed by diverse molecules within the binding pocket

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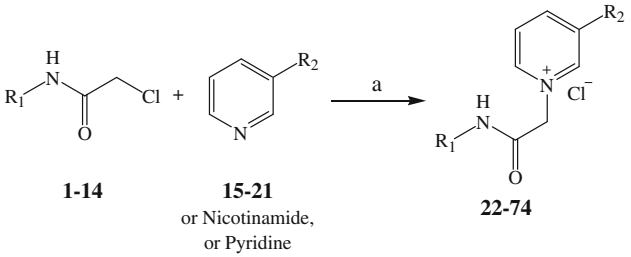
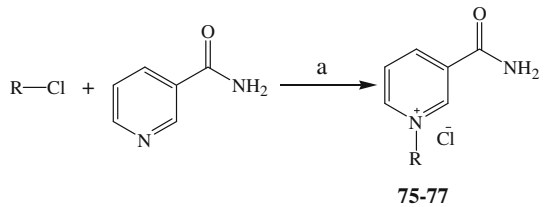


Scheme 3 Synthesis of the pyridinium acetamide derivatives. (*a*) Fusion with nicotinamide (**22**–**34**), pyridine (**35**–**44**), and nicotinyl derivatives (**45**–**74**)



Scheme 4 Synthesis of simple pyridinium derivatives. (*a*) Fusion in oil path at 150–160°C for 10 min (**75**–**77**)

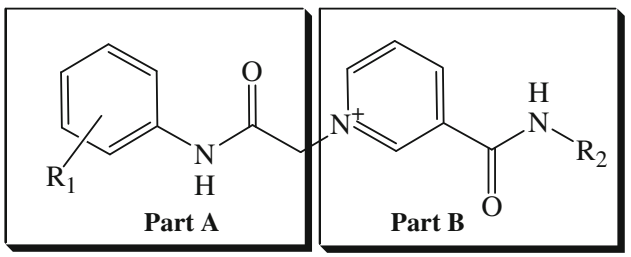


Fig. 3 General scaffold of synthesized compounds **22**–**77** showing two parts explored through variable chemical substituents

of Hsp90α. Interestingly, in cases were the aryl-acetamido of part A (Fig. 3) is substituted with a contradicting combination of large and electron-withdrawing fragments, the presence of unsubstituted *meta*-acetamido group on the pyridinium moiety (i.e., nicotinamide in part B, Fig. 3) seems to have no influence on bioactivity, e.g., **32** vs. **42** and **33** vs. **44**.

On the other hand, introduction of bulky substituents on the nicotinamidic $CONH_2$ seems to have detrimental effects on bioactivity, e.g., **72**, **66**, **58**, **55**, and **46** compared with **27**; also **69**, **64**, **61**, and **45** compared with **31**. This trend is probably related to steric factors resulting from the bulky substitutions at the pyridinium fragment (part B, Fig. 3) of the molecules. However, this trend seems to break in cases of *p*-tolyl fragment at part A (Fig. 3), i.e., aromatic substituents on part B improves the anti-Hsp90α bioactivities of *p*-tolyl derivatives, e.g., **30** compared with **49** and **67**. This behavior can also be explained by variable binding modes.

Docking of active synthetic compounds and comparison with crystallographic complex of Hsp90α

To probe the behavior of high-ranking inhibitors, we compared the docked poses of thiamine, **23**, **28**, **27**, and **38**. The docking experiments were performed employing Ligandfit docking engine via default docking parameters and consensus scoring function (Venkatachalam *et al.*, 2003; Vieth *et al.*, 1998). These settings were validated by the close resemblance between the docked pose of compound BXZ1, generated by LigandFit docking simulation, and the crystallographic structure of this inhibitor within Hsp90α (PDB code: 3BM9, resolution 1.6 Å) (Gopalsamy *et al.*, 2008), as shown in Fig. 1.

Figure 1c shows the docked pose of thiamine into Hsp90α. Clearly, the docking experiment suggest that thiamine docks with Hsp90α ATP pocket through the following interactions: (i) The terminal alcoholic OH of thiamine is hydrogen bonded to the NH_3 of LYS58 while the opposing pyrimidinyl amine is involved in hydrogen-bonding interaction with the carboxylate group of ASP93. (ii) The pyrimidinyl methyl group seems to be hydrophobically involved with the side chains of PHE138 and LEU107. (iii) The thiazolinum ring is positioned within a pocket comprised of the hydrophobic side chains of MET98, ILE96 and ALA55. (iv) Similarly, the pyrimidine ring was fitted by the docking engine between the methylene linker of ASN51, methyl of THR184 and aromatic ring of PHE138.

The docked poses of **23** and **28** (IC_{50} = 16.4 μM, 13.2 μM, respectively, Fig. 4a, b) show close resemblance to that thiamine (Fig. 1c). (i) The *meta*-acetamido groups of the pyridinium fragments (nicotinamide) of **23** and **28** were docked at close proximity with the NH_3 of LYS58 suggesting mutual hydrogen-bonding interactions, while the acetamido linker of both molecules (part A, Fig. 3) seems to hydrogen bond with the OH and COOH of THR184 and ASP93, resepectively. (ii) The phenylethyl and *meta*-methyl fragments of **23** and **28** (Part A as in Fig. 3), respectively, were positioned close to the side chains of PHE138 and LEU107 suggesting corresponding hydrophobic interactions with the binding pocket. (iii) The pyridinium ring in both molecules (i.e., **23** and **28**) were positioned within the hydrophobic vicinity of the side chains of MET98, ILE96, and ALA55. (iv) Finally, the ethyl linker of **23** and *meta*-tolyl ring of **28** were docked close to the methyl of THR184 and the methylene linker of ASN51.

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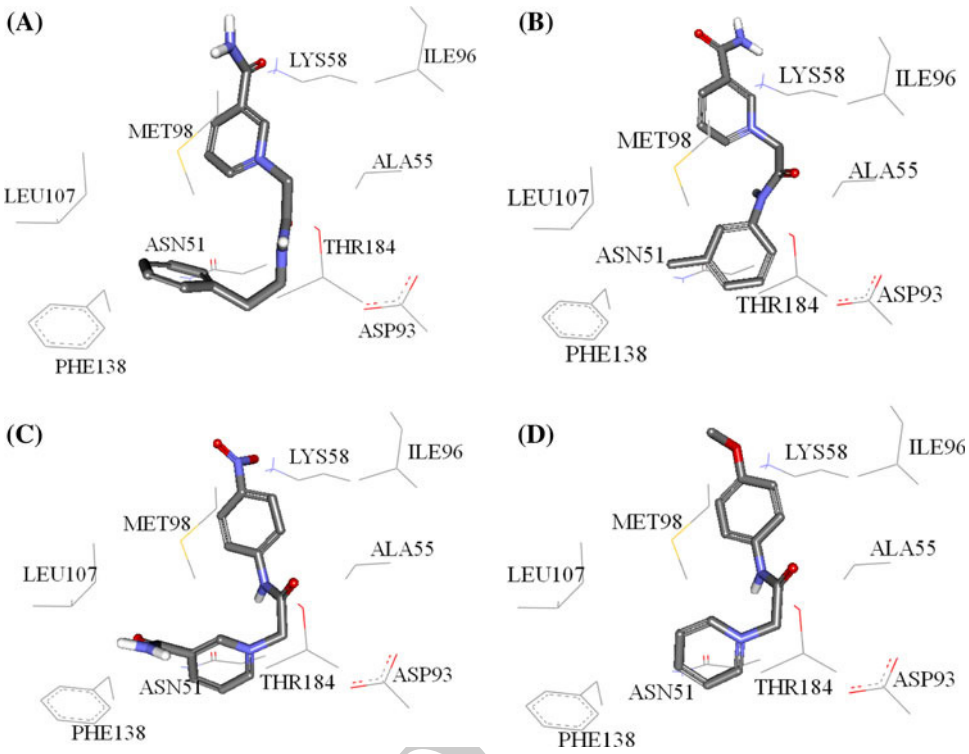

Fig. 4 **a** **23**, **b** **28**, **c** **27**, and **d** **38** docked into the same protein using LigandFit docking engine and consensus scoring function

On the other hand, compounds **27** and **38** (IC_{50} = 7.6 μM, 13.3 μM, respectively) seem to assume flipped poses in the binding pocket of Hsp90α (Fig. 4c, d). The *para*-nitro and *para*-methoxy groups of the molecules (i.e., in Part A, Fig. 3) were docked close to LYS58 suggesting mutual hydrogen bonding with its NH_3 group. While the *meta*-acetamido group of the pyridinium moiety of **27** (Part B, Fig. 3) is positioned close to the aromatic ring of PHE138 suggesting mutual π-stacking. The positively charged pyridinium rings of both **27** and **38** were closely docked to the carboxylate of ASP93 suggesting mutual electrostatic attraction, while the *para*-substituted aniline rings of both molecules were docked within the hydrophobic pocket constructed from the side chains of MET98, ALA55, and ILE96.

Conclusion

The current work shows through experimental and docking evidence that thiamine moderately inhibits Hsp90α, which explains, at least partially, the reported anticancer properties of thiamine. Furthermore, we synthesized closely related pyridinium-based compounds and showed they posess comparable anti-Hsp90α properties. This work opens the door for future optimization of new related analogues as potential potent Hsp90α inhibitors.

Experimental section

Synthetic procedures

Melting points were measured using Gallenkampf melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectrums were collected on a Varian Oxford NMR-300 spectrometer. High resolution mass spectrometry was performed using LC Mass Bruker Apex-IV mass spectrometer utilizing an electrospray interface. Infrared spectra were recorded using Shimadzu IR Affinity-1 spectrophotometer. The samples were analyzed as thin solid films using KBr pellets. Analytical thin layer chromatography (TLC) was carried out using pre-coated aluminum plates and visualized by UV light (at 254 and/or 360 nm). Elemental analysis was performed using Euro Vector elemental analyzer. Chemicals and solvents were used without further purification.

*Synthesis of the mono-chloromethyl-acetamido derivatives (**1–14**) (Scheme 1)*

To a magnetically stirred, ice-bathed, solution or suspension of the particular aromatic amine **1–14**, (1.0 equivalent) and triethylamine (2.0 equivalents) in dry acetone (25 ml), chloroacetylchloride (1.0 equivalent) in dry acetone (25 ml) was gradually added over 30 min. The reaction

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mixture was stirred at room temperature until TLC revealed complete consumption of the starting amine. Subsequently, the reaction mixture was poured slowly onto 100 ml of 5% aqueous sodium bicarbonate to neutralize the generated acid. The precipitated crude products were purified by recrystalization from acetone/water.

*N-Benzyl-2-chloro-acetamide (**1**)* This compound was prepared from benzylamine (1.0 ml, 9 mmol) to yield **1** as white powder (1.23 g, 66%); mp 95–96°C, IR (KBr): $\nu_{max} = 3278$, 1650, 1550 cm^{-1}, ^1H NMR (300 MHz, Acetone-d_6): $\delta = 4.12$ (s, 2H, CH_2), 4.45 (d, 2H, CH_2, $J = 6.3$ Hz), 7.2–7.32 (m, 5H, phenyl), 7.95 (s, 1H, $CONH_2$) ppm.; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 42.71$ (CH_2), 43.16 (CH_2), 127.2 (CH), 127.73 (2 × CH), 128.57 (2 × CH), 139.28 (C), 166.08 (C=O) ppm. HRMS-FAB *m/z* $[M + Na]^+$ calcd for $C_9H_{10}ClNNaO$: 206.03486, found: 206.03431.

*2-Chloro-N-phenethyl-acetamide (**2**)* This compound was prepared from phenylethylamine (1.0 ml, 8 mmol) to yield **2** as white powder (0.5 g, 32%); mp 68–69°C, IR (KBr): $\nu_{max} = 3351$, 1651, 1556 cm^{-1}, ^1H NMR (300 MHz, Acetone-d_6): $\delta = 2.84$ (t, 2H, CH_2, $J = 7.5$ Hz), 3.486 (t, 2H, CH_2, $J = 6.6$ Hz), 4.05 (s, 2H, CH_2), 7.25 (m, 5H, phenyl), 7.53 (s, 1H, CO–NH) ppm.; ^{13}C NMR (75 MHz, Acetone-d_6): $\delta = 43.12$ (CH_2), 41.62 (CH_2), 35.99 (CH_2), 126.87 (2 × CH), 129.05 (2 × CH), 129.39 (CH), 140.00 (C), 166.30 (C=O) ppm.; HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{10}H_{13}NOCl$: 198.06857, found: 198.07023.

*2-Chloro-N-phenyl-acetamide (**3**)* This compound was prepared from aniline (1.0 ml, 10 mmol) to yield **3** as white powder (1.7 g, 92%); mp 136–137°C, IR (KBr): $\nu_{max} = 3250$, 1690, 1610, 1552 cm-1, ^1H NMR (300 MHz, Acetone-d_6): $\delta = 4.23$ (s, 2H, CH_2), 7.1 (dd, 1H, phenyl, $J = 7.2$ Hz), 7.32 (dd, 2H, phenyl, $J = 7.8$ Hz), 7.67 (d, 2H, phenyl, $J = 7.8$ Hz), 9.40 (br s, 1H, CONH) ppm.; ^{13}C NMR (75 MHz, Acetone-d_6) $\delta = 43.5$ (CH_2), 119.90 (2 × CH), 124.32 (CH), 129.0 (2 × CH), 138.80 (C), 164.75 (C=O) ppm. HRMS-FAB *m/z* $[M + Na]^+$ calcd for $C_8H_8ClNNaO$: 192.01921, found: 192.01866.

*2-Chloro-N-(4-chloro-phenyl)-acetamide (**4**)* This compound was prepared from 4-chloro-aniline (1.0 g, 8 mmol) to yield **4** as pale grayish solid (1.5 g, 94%); mp 172–173°C (Decomp.), IR (KBr): $\nu_{max} = 3261$, 3201, 1665, 1602, 1551 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 4.24$ (s, 2H, CH_2), 7.35 (d, 2H, phenyl, $J = 8.7$ Hz), 7.70 (d, 2H, phenyl, $J = 9.0$ Hz), 9.53 (s, 1H, CO–NH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 43.43$ (CH_2), 121.38 (2 × CH), 128.94 (2 × CH), 128.673 (C), 137.693 (C), 164.89 (C=O); HRMS-FAB *m/z* $[M + Na]^+$ calcd for $C_8H_7Cl_2NNaO$: 225.98023, found: 225.97969.

*2-Chloro-N-(4-methoxy-phenyl)-acetamide (**5**)* This compound was prepared from *p*-anisidine (1.0 g, 8 mmol) to yield **5** as gray crystaline solid (0.8 g, 50%); mp 121–122°C, IR (KBr): $\nu_{max} = 3301$, 1660, 1552, 1502 cm^{-1}; ^1H NMR (300 MHz, Acetone-d_6): $\delta = 3.78$ (s, 3H, CH_3), 4.21 (s, 2H, CH_2), 6.90 (d, 2H, phenyl, $J = 9.0$ Hz), 7.58 (d, 2H, phenyl, $J = 9.3$ Hz), 9.27 (s, 1H, CO–NH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 43.87$ (CH_2), 55.44 (CH_3), 114.50 (2 × CH), 121.95 (2 × CH), 132.31 (C), 157.11 (C), 164.73 (C=O) ppm; HRMS-FAB *m/z* $[M + Na]^+$ calcd for $C_9H_{10}ClNNaO_2$: 222.02977, found: 222.02923.

*2-Chloro-N-(4-nitro-phenyl)-acetamide (**6**)* This compound was prepared from *p*-nitroaniline (1.0 g, 7.2 mmol) to yield **6** as yellow crystalline solid (1.1 g, 71.5%); mp 160–161°C, IR (KBr): $\nu_{max} = 3280$, 3210, 1690, 1640, 1610, 1562 cm^{-1}, ^1H NMR (300 MHz, Acetone-d_6): $\delta = 4.33$ (s, 2H, CH_2), 7.94 (d, 2H, phenyl, $J = 9.0$ Hz), 8.25 (d, 2H, phenyl, $J = 9.0$ Hz), 9.97 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 43.46$ (CH2), 119.52 (2 × CH), 125.00 (2 × CH), 143.10 (C), 145.234 (C), 164.29 (C=O); HRMS-FAB *m/z* $[M + Na]^+$ calcd for $C_8H_7ClN_2NaO_3$: 237.00429, found: 237.00374.

*2-Chloro-N-m-tolyl-acetamide (**7**)* This compound was prepared from *m*-toluidine (1.0 ml, 9 mmol) to yield **7** as pale white powder (0.95 g, 56%); mp 96–97°C, IR (KBr): $\nu_{max} = 3303$, 1686, 1601, 1545 cm^{-1}, ^1H NMR (75 MHz, Acetone-d_6): $\delta = 2.30$ (s, 3H, CH_3), 4.22 (s, 2H, CH_2), 6.94 (d, 1H, phenyl, $J = 7.5$ Hz), 7.20 (dd, 1H, phenyl, $J = 8.1$ Hz), 7.45 (s, 1H, phenyl), 7.49 (d, 1H, phenyl, $J = 6.6$ Hz), 9.31 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, Acetone-d_6): $\delta = 20.86$ (CH_3), 43.53 (CH_2), 116.99 (CH), 120.40 (CH), 125.02 (CH), 128.82 (CH), 138.67 (C), 138.75 (C), 164.60 (C=O) ppm; HRMS-FAB *m/z* $[M + Na]^+$ calcd for $C_9H_{10}ClNNaO$: 206.03486, found: 206.03431.

*2-Chloro-N-o-tolyl-acetamide (**8**)* This compound was prepared from *o*-toluidine (1.0 ml, 9 mmol) to yield **8** as a white powder (1.3 g, 76%); mp 111–112°C, IR (KBr): $\nu_{max} = 3264$, 1658, 1545 cm^{-1}, ^1H NMR (300 MHz, Acetone-d_6): $\delta = 2.26$ (s, 3H, CH_3), 4.30 (s, 2H, CH_2), 7.12 (d, 1H, phenyl, $J = 7.5$ Hz), 7.21 (dd, 2H, phenyl, $J = 7.5$ Hz), 7.57 (d, 1H, phenyl, $J = 7.8$ Hz), 9.13 (s, 1H, CO–NH) ppm; ^{13}C NMR (75 MHz, Acetone-d_6): $\delta =$ 17.30 (CH_3), 43.29 (CH_2), 124.66 (CH), 125.93 (CH), 126.39 (CH), 130.65 (CH), 131.75 (C), 135.98 (C), 165.20

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(C=O) ppm; HRMS-FAB *m/z* $[M + Na]^+$ calcd for $C_9H_{10}ClNNaO$: 206.03486, found: 206.03431.

2-Chloro-N-p-tolyl-acetamide ***(9)*** This compound was prepared from *p*-toluidine (1.0 g, 9 mmol) to yield **9** as a pale white powder (1.4 g, 82%); mp 166–167°C, IR (KBr): $\nu_{max} = 3254, 1660, 1602, 1545, 1504\ cm^{-1}$, ^1H NMR (300 MHz, Acetone-d_6): $\delta = 2.24$ (s, 3H, CH_3), 4.24 (s, 2H, CH_2), 7.12 (d, 2H, phenyl, $J = 8.4$ Hz), 7.46 (d, 2H, phenyl, $J = 8.1$ Hz), 10.207 (s, 1H, CO–NH) ppm; ^{13}C NMR (75 MHz, Acetone-d_6): $\delta = 21.15$ (CH_3), 44.27 (CH_2), 120.03 (2 × CH), 129.93 (2 × CH), 133.52 (C), 136.65 (C), 165.059 (C=O) ppm; HRMS-FAB *m/z* $[M + Na]^+$ calcd for $C_9H_{10}ClNNaO$: 206.03486, found: 206.03431.

2-Chloro-N-(4-sulfamoyl-phenyl)-acetamide ***(10)*** This compound was prepared from sulfanilamide (1.0 g, 5.8 mmol) to yield **10** as white crystaline solid (1.1 g, 76%); mp 222–223°C, IR (KBr): $\nu max = 3320, 3201, 1695, 1602, 1545\ cm^{-1}$, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 4.29$ (s, 2H, CH_2), 6.53 (s, 2H, SO_2NH_2), 7.85 (s, 4H, phenyl), 9.73 (s, 1H, CO–NH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 43.91$ (CH_2), 119.90 (2 × CH, phenyl), 127.82 (2 × CH), 140.00 (C), 142.36 (C), 165.70 (C=O) ppm; HRMS-FAB *m/z* $[M + Na]^+$ calcd for $C_8H_9ClN_2NaO_3S$: 270.99201, found: 270.99146.

2-Chloro-N-[(4-Acetylsulfamoyl)-phenyl]-acetamide ***(11)*** This compound was prepared from sulfacetamide (1.0 g, 4.7 mmol) to yield **11** as white crystaline solid (1.3 g, 96%); mp 237–238°C, IR (KBr): $\nu_{max} = 3300, 3100, 1686, 1601, 1552\ cm^{-1}$, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 1.86$ (s, 3H, CH_3), 4.22 (s, 2H, CH_2), 7.82 (m, 4H, phenyl), 11.83 (s, 1H, CONH), 12.2 (br s, CO–NH–SO_2) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 23.41$ (CH_3), 43.88 (CH_2), 119.41 (2 × CH), 129.50 (2 × CH), 134.32 (C), 143.47 (C), 165.94 (C=O), 169.19 (C=O) ppm; HRMS-FAB *m/z* $[M + Na]^+$ calcd for $C_{10}H_{11}ClN_2NaO_4S$: 313.00257, found: 313.00203.

N-(4-Guanido-sulfonyl-phenyl)-2-chloro-acetamide ***(12)*** This compound was prepared from sulfaguanide (1.0 g, 4.7 mmol) to yield **12** as white powder (1.2 g, 88%); mp 170–171°C, IR (KBr): $\nu_{max} = 3500, 3415, 3340, 1694, 1640, 1542\ cm^{-1}$, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 4.23$ (s, 2H, CH_2), 6.65 (s, 4H, guanido), 7.68 (m, 4H, phenyl), 11.50 (s, 1H, CONH) ppm; ^{13}C NMR (300 MHz, DMSO-d_6): $\delta = 43.98$ (CH_2), 119.405 (2 × CH), 127.26 (2 × CH), 140.10 (C), 141.40 (C), 158.63 (C = N), 165.68 (C=O) ppm; HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_9H_{12}ClN_4O_3S$: 291.03186, found: 291.03132.

2-Chloro-N-(4-methylsulfanyl-phenyl)-acetamide ***(13)*** This compound was prepared from 4-methylthioaniline (1.0 ml, 8 mmol) to yield **13** as a pale gray powder (1.35 g, 87%); mp 140–142°C, IR (KBr): $\nu_{max} = 3333, 3186, 1658, 1589\ cm^{-1}$, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 2.41$ (s, 3H, CH_3), 4.20 (s, 2H, CH_2), 7.21 (d, 2H, phenyl, $J = 8.4$ Hz), 7.52 (d, 2H, phenyl, $J = 8.1$ Hz), 10.26 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 16.04$ (CH_3), 44.20 (CH_2), 120.72 (2 × CH), 127.71 (2 × CH), 133.26 (C), 136.55 (C), 165.17 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_9H_{10}NOSCl$: 215.01716, found: 215.09090.

N-allyl-2-chloro-acetamide ***(14)*** This compound was prepared from allyl amine (1.0 ml, 13.3 mmol), extracted with 50 ml ethyl acetate three times, and dried by 10 g of $MgSO_4$ to yield **14** as yellowish viscous liquid (0.6 g, 36%); IR (KBr): $\nu_{max} = 3287, 1690, 1551\ cm^{-1}$, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 3.69$ (d, 2H, $J = 4.8$ Hz), 4.25 (s, 2H, CH_2), 5.03 (dd, 1H, Allylic CH, $J = 3.0$ Hz, 1.5 Hz), 5.21 (dd, 1H, Allylic CH, $J = 3.0$ Hz, 1.5 Hz), 5.76 (ddd, 1H, Allylic CH, $J = 5.4$ Hz, 10.2 Hz, 12.0 Hz), 9.63 (s, 1H, CO–NH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 42.025$ (CH_2), 43.90 (CH_2), 116.50 (CH_2), 127.87 (CH), 163.39 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_9H_{12}ClN_4O_3S$: 156.01921, found: 156.12670.

Synthesis of N-substituted-nicotinamide derivatives ***(15–21)*** *(Scheme 2)*

To stirred ice-bathed neat oxalyl chloride (5 ml, 58 mmol) nicotinic acid (5 g, 40 mmol) was added to form thick slurry. The reaction mixture was left at room temperature for one hour during which excess oxalyl chloride was allowed to evaporate in fume hood to yield whitish powder. Subsequently, the particular amine (1.0 equivalent, neat) was added under vigorous stirring to the resulting powder under ice bath conditions. The reaction was subsequently warmed to room temperature and stirred for 15 min. The reaction was terminated by quenching with 5% aqueous $NaHCO_3$ solution (100 ml). The resulting crude precipitate was filtered and recrystallized from acetone/water to yield compounds **15**–**21**.

N-Phenyl nicotinamide ***(15)*** This compound was prepared from aniline (5.0 ml, 52 mmol) to yield **15** as white powder (1.15 g, 71%); mp 122–123°C, IR (KBr): $\nu_{max} = 3350, 1648, 1601, 1532\ cm^{-1}$, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 7.10$ (d, 1H, pyridinium, $J = 6.9$ Hz), 7.34 (d, 2H, phenyl, $J = 6.9$ Hz), 7.53 (dd, 1H, pyridinium, $J = 5.1$ Hz), 7.75 (d, 2H, phenyl, $J = 7.5$ Hz), 8.27 (dd, 1H, pyridinium, $J = 7.5$ Hz), 8.73 (d, 2H, pyridinium,

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$J = 4.8$ Hz), 9.09 (s, 1H, pyridinium), 10.44 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta =$ 121.017 (CH), 124.17 (2 × CH), 124.65 (2 × CH), 129.38 (CH), 131.28 (C), 136.15 (CH), 139.52 (C), 149.36 (CH), 152.77 (CH), 164.74 (C=O) ppm; HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{12}H_{11}N_2O$: 199.08714, found: 199.08659.

N-m-Tolyl nicotinamide (***16***) This compound was prepared from *m*-toluidine (5.0 ml, 46.7 mmol) to yield **16** as pale white powder (1.25 g, 72%); mp 123–124°C, IR (KBr): $\nu_{max} = 3270, 1678, 1601, 1533$ cm^{-1}, ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 2.30$ (s, 3H, CH_3), 6.98 (d, 1H, phenyl, $J = 6.6$ Hz), 7.26 (br s, 1H, phenyl), 7.38 (d, 2H, phenyl, $J = 7.8$ Hz), 8.35 (br s, 1H, pyridinium), 9.19 (br s, 1H, pyridinium), 9.69 (s, 1H, pyridinium), 11.07 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 21.86$ (CH_3), 118.38 (CH), 121.66 (CH), 126.07 (CH), 127.74 (CH), 128.26 (C), 129.38 (CH), 137.94 (C), 138.77 (C), 145.36 (CH), 147.39 (CH), 148.82 (CH), 163.88 (C=O) ppm; HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{13}H_{13}N_2O$: 213.10279, found: 213.10224.

N-Allyl-nicotinamide (***17***) This compound was prepared from allylamine (5.0 ml, 67 mmol) However, after quenching with 5% aqueous $NaHCO_3$ solution (100 ml), the aqueous layer was extracted with ethylacetate (3 × 50 ml), dried over anhydrous $MgSO_4$ and evaporated in vacuo to yield **17** (0.85 g, 64%) as yellowish viscous liquid; IR (KBr): $\nu_{max} = 3294, 1651, 1543$ cm^{-1}, ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.90$ (m, 2H, allyl CH_2), 5.06 (dd, 1H, allyl, $J = 3.3$, 10.2 Hz), 5.15 (dd, 1H, allyl, $J = 3.6$, 15.3 Hz), 5.85 (ddd, 1H, CH, allyl, $J = 5.1$, 10.5, 15.9 Hz), 7.45 (dd, 1H, $J = 3.3$, 4.8 Hz), 8.18 (dd, 1H, pyridinium, $J = 1.8$, 6.0 Hz), 8.65 (dd, 1H, pyridinium, $J = 3.0$, 4.8 Hz), 8.86 (br s, 1H, CONH), 9.01 (s, 1H, pyridinium) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 42.18$ (CH_2), 116.03 (CH_2), 124.07 (CH), 130.53 (C), 135.69 (CH), 135.62 (CH), 149.06 (CH), 152.44 (CH), 165.34 (C=O); HRMS-FAB *m/z* $[M + Na]^+$ calcd for $C_9H_{10}N_2NaO$: 185.06908, found: 185.06853.

N-(4-Methoxy-phenyl)-nicotinamide (***18***) This compound was prepared from *p*-anisidine/dichloromethane solution (1.0 g/5 ml, 8 mmol) to yield **18** as gray crystalline solid (0.97 g, 52%); mp 145–146°C, IR (KBr): $\nu_{max} = 3333, 3124, 1643, 1589$ cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.74$ (s, 3H, OCH_3), 6.95 (d, 2H, phenyl, $J = 9.0$ Hz), 7.54 (dd, 1H, pyridinium, $J = 0.9$, 5.7 Hz), 7.67 (d, 2H, phenyl, $J = 9.0$ Hz), 8.29 (dd, 1H, pyridinium, $J = 1.5$, 3.0 Hz), 8.75 (d, 1H, pyridinium, $J = 5.1$ Hz), 9.11 (s, 1H, pyridinium), 10.35 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 55.84$ (CH_3, OCH_3), 114.48 (2 × CH), 122.7 (2 × CH), 124.15 (CH), 131.33 (C), 132.564 (C), 136.04 (CH), 149.31 (CH), 152.64 (CH), 156.44 (C), 164.282 (C=O); HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{13}H_{13}N_2O_2$: 229.09770, found: 229.09715.

N-(4-Nitro-phenyl)-nicotinamide (***19***) This compound was prepared from 4-nitro-aniline/dichloromethane solution (1.0 g/5 ml, 7.2 mmol) to yield **19** as yellow crystalline solid (1.5 g, 85.7%); mp 147–148°C, IR (KBr): $\nu_{max} = 3479, 3356, 3217, 1589$ cm^{-1}, ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 7.90$ (d, 2H, phenyl, $J = 8.7$ Hz), 8.30 (d, 2H, phenyl, $J = 8.7$ Hz), 8.37 (dd, 1H, pyridinium, $J = 7.5$ Hz), 9.21 (d, 1H, pyridinium, $J = 6.0$ Hz), 9.28 (d, 1H, pyridinium, $J = 8.4$ Hz), 9.77 (s, 1H, pyridinium), 11.85 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 121.43$ (2 × CH), 125.57 (CH), 127.7 (2 × CH), 132.01 (C), 143.92 (C), 145.13 (C), 145.64 (CH), 149.20 (CH), 156.35 (C), 162.99 (C=O) ppm, HRMS-FAB *m/z* $[M\text{-}H]^+$ calcd for $C_{12}H_8N_3O_3$: 242.05657, found: 242.05711.

N-(4-Chloro-phenyl)-nicotinamide (***20***) This compound was prepared from 4-chloro-aniline/dichloromethane solution (1.0 g/5 ml, 8 mmol) to yield **20** as pale white crystalline solid (1.4 g, 74%); mp 169–170°C, IR (KBr): $\nu_{max} = 3240, 3178, 1689, 1597, 1535$ cm^{-1}, ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 7.42$ (d, 1H, phenyl, $J =$ 9.0 Hz), 7.79 (d, 2H, phenyl, $J = 9.0$ Hz), 7.89 (dd, 1H, pyridinium, $J = 3.6$ Hz), 8.27 (dd, 1H, pyridinium, $J = 1.5$, 3.6 Hz), 8.75 (d, 1H, pyridinium, $J = 7.8$ Hz), 9.085 (s, 1H, pyridinium), 10.56 (s, 1H, CO–NH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta =$ 122.53 (2 × CH), 124.20 (2 × CH), 129.38 (CH), 131.05 (C), 136.18, (CH), 138.51 (C), 149.38 (CH), 152.92 (CH), 159.18 (C), 164.85 (C=O) ppm; HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{12}H_9N_2OCl$: 233.04816, found: 233.04762.

N-(4-Methyl-sulfanyl-phenyl)-nicotinamide (***21***) This compound was prepared from 4-methylthioaniline/dichloromethane solution (1.0 g/5 ml, 8 mmol) to yield **21** as pale white crystalline solid (1.23 g, 61.5%); mp 165–166°C, IR (KBr): $\nu_{max} = 3356, 3171, 1651, 1589$ cm^{-1}, ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 2.43$ (s, 3H, CH_3), 7.25 (d, 2H, phenyl, $J = 8.7$ Hz), 7.53 (dd, 1H, pyridinium, $J = 4.8$, 7.5 Hz), 7.70 (d, 2H, phenyl, $J = 8.7$ Hz), 8.25 (d, 1H, pyridinium, $J = 7.8$ Hz), 8.72 (d, 1H, pyridinium, $J = 4.5$ Hz), 9.07 (s, 1H, pyridinium), 10.40 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 16.06$ (CH_3), 121.68 (2 × CH), 124.15 (2 × CH), 127.52 (CH), 131.19 (C), 133.46 (C), 136.1 (CH), 136.93 (C), 149.31 (CH), 152.76 (CH), 164.58 (C=O) ppm; HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{13}H_{13}N_2OS$: 245.07486, found: 245.07299.

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Synthesis of the pyridinium cationic derivatives (22–77) (Schemes 3, 4)

To magnetically stirred neat pyridine, nicotinamide, or *N*-substituted nicotinamide **15–21** (3.0 equivalents) heated to 150–160°C, the particular mono-chloromethyl-acetamide derivative **1–14** or aryl alkyl chloride (benzyl, phenylethyl or ethyloxycarbonylmethyl chloride) was added neat (1.0 equivalent). The reaction mixture was stirred at 150–160°C for 10–15 min then cooled to room temperature. The resulting solid mass was suspended in dry acetone (20 ml) and stirred at room temperature for one hour then filtered. The residues were further washed with acetone (2 × 20 ml) to yield pyridinum derivatives **22–77**.

*1-(Benzylcarbamoyl-methyl)-3-carbamoyl-pyridinium chloride (**22**)* This compound was prepared from **1** (0.25 g, 1.4 mmol) and nicotinamide (0.5 g, 4 mmol) to yield **22** as pale white powder (0.298 g, 71.5%); mp 226–227°C (Decomp.), IR (KBr): $\nu_{max} = 3310$, 1710, 1540 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 4.35$ (d, 2H, CH_2, $J = 6$ Hz), 5.67 (s, 2H, CH_2), 7.30 (m, 5H, phenyl), 8.22 (s, 1H, CO–NH_2), 8.29 (m, 1H, pyridinium), 8.95 (s, 1H, CO–NH_2), 9.15(s, 1H, CO–NH), 9.16 (m, 1H, pyridinium), 9.52 (dd, 1H, pyridinium, $J = 5.7$ Hz), 9.63 (s, 1H, pyridinium) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 43.26$ (CH_2), 62.31 (CH_2), 127.71 (2 × CH), 127.85 (2 × CH), 128.11 (C), 129.02 (CH), 133.75 (C), 139.11 (CH), 144.76 (CH), 147.20 (CH), 148.59 (CH), 163.41 (C=O), 165.0 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{15}H_{16}N_3O_2$: 270.12425, found: 270.12370.

*3-Carbamoyl-1-(phenethylcarbamoyl-methyl)-pyridinium chloride (**23**)* This compound was prepared from **2** (0.25 g, 1.3 mmol) and nicotinamide (0.5 g, 4 mmol) to yield **23** as yellowish white solid (0.32 g, 79%); mp 207–208°C (Decomp.), IR (KBr): $\nu_{max} = 3301$, 1705, 1656, 1607, 1558 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 2.76$ (t, CH_2, $J = 7.5$ Hz), 3.33 (t, CH_2, $J = 7.5$ Hz), 5.58 (s, CH_2), 7.2 (m, 5 H, phenyl), 8.23 (s, 1H, $CONH_2$), 8.27 (d, 1H, pyridinium, $J = 8.1$ Hz), 8.95 (s, 1H, $CONH_2$), 9.15 (s, 1H, CONH), 9.16 (d, 2H, pyridinium, $J = 6.9$ Hz), 9.60 (s, 1H, pyridinium) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 35.6$ (CH_2), 41.51 (CH_2), 62.37 (CH_2), 126.92 (CH), 127.85 (CH), 129.09 (2 × CH), 129.38 (2 × CH), 133.75 (CH), 139.77 (C), 144.76 (CH), 147.37 (CH), 148.52 (C), 163.40 (C=O), 164.78 (C=O) ppm.; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{16}H_{18}N_3O_2$: 284.13990, found: 284.13935; Anal. Calcd for $C_{16}H_{18}N_3O_2Cl$; C: 60.1, H: 5.7, N: 13.1, found: C: 60.49, H: 5.7, N: 13.41.

*3-Carbamoyl-1-phenylcarbamoyl-methyl-pyridinium chloride (**24**)* This compound was prepared from **3** (0.25 g, 1.5 mmol) and nicotinamide (0.5 g, 4 mmol) to yield **24** as white powder (0.33 g, 76%); mp 236–237°C (Decomp.), IR (KBr): $\nu_{max} = 3304$, 1701, 1646, 1598 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 5.86$ (s, 2H, CH_2), 7.30 (dd, 1H, phenyl, $J = 7.5$ Hz), 7.63 (dd, 2H, phenyl, $J = 7.8$ Hz), 8.24 (s, 1H, CO–NH_2), 8.32 (dd, 1H, pyridinium, $J = 6.6$ Hz), 8.94 (s, 1H, CO–NH_2), 9.172 (d, 1H, pyridinium, $J = 5.7$ Hz), 9.28 (d, 1H, pyridinium, $J = 5.7$ Hz), 9.69 (s, 1H, pyridinium), 11.59 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 63.05$ (CH_2), 119.85 (CH), 124.56 (CH), 127.89 (2 × CH), 129.57 (2 × CH), 133.76 (C), 139.09 (C), 144.87 (CH), 147.37 (CH), 148.71 (CH), 163.45 (C=O) 163.73 (C=O) ppm. HRMS-FAB *m/z* $[M]^+$ calcd for $C_{14}H_{14}N_3O_2$: 256.10860, found: 256.10805; Anal. Calcd for $C_{14}H_{14}N_3O_2$: C: 57.6, H: 4.8, N: 14.4 found: C: 57.1, H: 4.82, N: 14.33.

*3-Carbamoyl-1-[(4-chloro-phenylcarbamoyl)-methyl]-pyridinium chloride (**25**)* This compound was prepared from **4** (0.25 g, 1.2 mmol) and nicotinamide (0.5 g, 4 mmol) to yield **25** as pale white solid (0.34 g, 85%); m.p 279–280°C (Decomp.), IR (KBr): $\nu_{max} = 3330$, 1703, 1651, 1606, 1545 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 5.82$ (s, CH_2), 7.3 (d, 2H, phenyl, $J = 8.5$ Hz), 7.6 (d, 2H, phenyl, $J = 8.5$ Hz), 8.31 (d, 1H, pyridinium, $J = 7.2$ Hz), 8.18 (s, 1H, $CONH_2$), 8.84 (s, 1H, $CONH_2$), 9.14 (d, 1H, pyridinium, $J = 8.1$ Hz), 9.22 (d, 1H, pyridinium, $J = 6$ Hz), 9.64 (s, 1H, pyridinium), 11.62 (s, 1H, CO–NH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 63.046$ (CH_2), 121.45 (2 × CH), 128.15 (C), 127.88 (2 × CH), 129.49 (C), 133.86 (C), 138.1 (C), 148.74 (CH), 147.37 (CH), 144.87 (CH), 163.40 (C=O), 163.93 (C=O); HRMS-FAB *m/z* $[M]^+$ calcd for $C_{14}H_{13}ClN_3O_2$: 290.06963, found: 290.06908.

*3-Carbamoyl-1-(4-methoxy-phenylcarbamoyl-methyl)-pyridinium chloride (**26**)* This compound was prepared from **5** (0.25 g, 1.3 mmol) and nicotinamide (0.5 g,4 mmol) to yield **26** as grayish white solid (0.314 g, 78%); mp 255–256°C (Decomp.), IR (KBr): $\nu_{max} = 3451$, 3305, 1709, 1640, 1610, 1542 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 3.706$ (s, 3H, CH_3), 5.764 (s, 2H, CH_2), 6.890 (d, 2H, phenyl, $J = 8.7$ Hz), 7.54 (d, 2H, phenyl, $J = 9.0$ Hz), 8.16 (s, 1H, CO–NH_2), 8.31 (dd, 1H, pyridinium, $J = 7.5$ Hz), 8.80 (s, 1H, CO–NH_2), 9.12 (d, 1H, pyridinium, $J = 8.1$ Hz), 9.21 (d, 1H, pyridinium, $J = 5.7$ Hz), 9.62 (s, 1H, pyridinium), 11.11 (s, 1H, CO–NH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 55.88$ (CH_3, OCH_3), 62.96 (CH_2), 114.71 (2 × CH), 121.44 (2 × CH), 127.89 (CH), 132.13 (C), 133.91 (C), 144.80 (CH), 147.26 (CH), 148.68 (CH), 156.325 (C), 163.088 (C=O), 163.432 (C=O); HRMS-FAB *m/z* $[M]^+$ calcd for $C_{15}H_{16}N_3O_3$: 286.11917, found: 286.11862.

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*3-Carbamoyl-1-[(4-nitro-phenylcarbamoyl)-methyl]-pyridinium chloride (**27**)* This compound was prepared from **6** (0.25 g, 1.1 mmol) and nicotinamide (0.5 g, 4 mmol) to yield **27** as yellow fine solid (0.302 g, 77%); mp 269–270°C (Decomp.), IR (KBr): $\nu_{max} = 3451$, 3303, 1705, 1646, 1601, 1555 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 5.87$ (s, 2H, CH_2), 7.90 (d, 2H, $J = 8.7$ Hz, phenyl), 8.23 (d, 2H, $J = 8.7$ Hz, phenyl), 8.14 (s, 1H, $CONH_2$), 8.32 (s, 1H, $CONH_2$), 8.75 (s, 1H, pyridinium), 9.21 (m, 2H, pyridinium), 9.63 (s, 1H, pyridinium), 12.1 (br s, 1H, CONH) ppm, ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 63.27$ (CH_2), 119.82 (2 × CH), 125.74 (2 × CH), 127.92 (CH), 133.98 (C), 143.4 (C), 144.94 (CH), 145.20 (C), 147.45 (CH), 148.74 (CH), 163.40 (C=O), 164.96 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{14}H_{13}N_4O_4$: 301.09368, found: 301.09313.

3-Carbamoyl-1-(m-tolylcarbamoyl-methyl)-pyridinium chloride (28) This compound was prepared from **7** (0.25 g, 1.4 mmol) and nicotinamide (0.5 g, 4 mmol) to yield **28** as white solid (0.35 g, 84%); mp 256–257°C (Decomp.), IR (KBr): $\nu_{max} = 3301$, 1701, 1590, 1544 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 2.23$ (s, 3H, CH_3), 5.83 (s, 2H, CH_2), 6.88 (d, 1H, $J = 7.5$ Hz, phenyl), 7.17 (dd, 1H, phenyl, $J = 7.8$ Hz), 7.40 (d, 1H, phenyl, $J = 8.4$ Hz), 7.47 (s, 1H, phenyl), 8.23 (s, 1H, CO–NH_2), 8.31 (dd, 1H, pyridinium, $J = 6.3$ Hz), 8.91 (s, 1H, CO–NH_2), 9.24 (d, 1H, pyridinium, $J = 6$ Hz), 9.18 (d, 1H, pyridinium, $J = 6.9$ Hz), 9.63 (s, 1H, pyridinium), 11.33 (s, 1H, CO–NH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 21.87$ (CH_3), 63.06 (CH_2), 117.05 (CH), 120.36 (CH), 125.26 (CH), 127.87 (CH), 129.40 (CH), 133.78 (C), 138.77, (C), 138.99 (C), 144.82 (CH), 147.35 (CH), 148.69 (CH) 163.43 (C=O), 163.66 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{15}H_{16}N_3O_2$: 270.12425, found: 270.12370; Anal. Calcd for $C_{15}H_{16}N_3O_2Cl$; C: 58.9, H: 5.3, N: 13.7, found: C: 58.5, H: 5.28, N: 13.84.

*3-Carbamoyl-1-(o-tolylcarbamoyl-methyl)-pyridinium chloride (**29**)* This compound was prepared from **8** (0.25 g, 1.4 mmol) and nicotinamide (0.5 g, 4 mmol) to yield **29** as pale white solid (0.31 g, 74.4%); mp 257–258°C (Decomp.), IR (KBr): $\nu_{max} = 3352$, 3246, 1698, 1601, 1554 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 2.29$ (s, 3H, CH_3), 5.87 (s, 2H, CH_2), 7.06–7.23 (m, 3H, phenyl), 7.41 (d, 1H, phenyl, $J = 7.8$ Hz), 8.18 (s, 1H, CO–NH_2), 8.30 (dd, 1H, pyridinium, $J = 7.5$ Hz), 8.88 (s, 1H, $CONH_2$), 9.14 (d, 1H, pyridinium, $J = 8.1$ Hz), 9.26 (d, 1H, pyridinium, $J = 6.0$ Hz), 9.69 (s, 1H, pyridinium), 10.52 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 18.73$ (CH_3), 62.87 (CH_2), 125.57 (CH), 126.39 (CH), 126.65 (CH), 127.90 (CH), 131.14 (CH), 132.53 (C), 133.88 (C), 136.04 (C) 144.84 (CH), 147.28 (CH), 148.65 (CH), 163.44 (C=O), 164.02 (C=O); HRMS-FAB *m/z* $[M]^+$ calcd for $C_{15}H_{16}N_3O_2$: 270.12425, found: 270.12370.

3-Carbamoyl-1-(p-tolylcarbamoyl-methyl)-pyridinium chloride (30) This compound was prepared from **9** (0.25 g, 1.4 mmol) and nicotinamide (0.5 g, 4 mmol) to yield **30** as pale white powder (0.321 g, 77%); mp 265–266°C (Decomp.), IR (KBr): $\nu_{max} = 3248$, 1701, 1652, 1598, 1545 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 2.22$ (s, 3H, CH_3), 5.79 (s, 2H, CH_2), 7.12 (dd, 1H, phenyl, $J = 8.1$ Hz), 7.53 (d, 2H, phenyl, $J = 8.4$ Hz), 8.20 (s, 1H, CO–NH_2), 8.31 (dd, 1H, pyridinium, $J = 6.3$ Hz), 8.86 (s, 1H, CO–NH_2), 9.24 (d, 1H, pyridinium, $J = 6$ Hz), 9.15 (d, 1H, pyridinium, $J = 7.8$ Hz), 9.64 (s, 1H, pyridinium), 11.25 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 21.15$ (CH_3), 63.02(CH_2), 119.83 (2 × CH), 127.87 (CH), 129.97 (2 × CH), 133.56 (C), 133.85 (C), 136.54 (C), 144.8 (CH), 147.33 (CH), 148.7 (CH), 163.38 (C=O), 163.45 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{15}H_{16}N_3O_2$: 270.12425, found: 270.12370.

*3-Carbamoyl-1-{[(4-sulfamoyl)-phenylcarbamoyl]-methyl}-pyridinium chloride (**31**)* This compound was prepared from **10** (0.25 g, 1 mmol) and nicotinamide (0.5 g, 4 mmol) to yield **31** as white solid (0.28 g, 75%); mp 229–230°C (Decomp.), IR (KBr): $\nu_{max} = 3320$, 1701, 1650, 1604, 1551 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 5.84$ (s, 2H, CH_2), 7.34 (s, 2H, SO_2NH_2), 7.82 (m, 4H, phenyl), 8.21 (s, 1H, $CONH_2$), 8.32 (dd, 1H, pyridinium, $J = 7.5$ Hz), 8.82 (s, 1H, $CONH_2$), 9.13 (d, 1H, pyridinium, $J = 8.1$), 9.22 (d, 1H, pyridinium, $J = 5.7$ Hz), 9.63 (s, 1H, pyridinium), 11.737 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 63.27$ (CH_2), 119.52 (2 × CH), 127.54 (2 × CH), 127.90 (C), 133.98 (C), 139.64 (CH), 141.91 (C), 144.87 (CH), 147.45 (CH), 148.74 (CH), 163.46 (C=O), 164.47 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{14}H_{15}N_4O_4S$: 335.08140, found: 335.08085.

*3-Carbamoyl-1-{[(4-acetylsulfamoyl)-phenylcarbamoyl]-methyl}-pyridinium chloride (**32**)* This compound was prepared from **11** (0.25 g, 0.9 mmol) and nicotinamide (0.5 g, 4 mmol) to yield **32** as white solid (0.31 g, 87%); mp 279–280°C (Decomp.), IR (KBr): $\nu_{max} = 3403$, 3119, 1701, 1652, 1603, 1551 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 1.91$ (s, 3H, CH_3), 5.86 (s, 2H, CH_2), 7.8 (m, 4H, phenyl), 8.22 (s, 1H, $CONH_2$), 8.34 (dd, 1H, pyridinium, $J = 7.8$), 8.82, (s, 1H, $CONH_2$), 9.13 (d, 1H, pyridinium, $J = 8.1$ Hz), 9.24 (d, 1H, pyridinium, $J = 6.3$ Hz), 9.64 (s, 1H, pyridinium), 11.83 (s, 1H, CONH), 12.2 (br s, CONH–SO_2) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 23.94$ (CH_3), 63.16 (CH_2), 119.54 (2 × CH), 127.93 (2 × CH), 129.28 (CH), 133.91 (CH), 134.38 (C), 143.5 (CH), 144.89 (C), 147.5 (CH), 148.76

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(C), 163.46 (C=O), 164.75 (C=O), 169.53 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{16}H_{17}N_4O_5S_1$: 377.09197, found: 377.09142; Anal. Calcd for $C_{16}H_{17}N_4O_5SCl$; C: 46.5, H: 4.2, N: 13.6, S: 7.8 found: C: 46.41, H: 4.57, N: 13.23, S: 8.72.

*3-Carbamoyl-1-{[4-(guanido-sulfonyl)-phenylcarbamoyl]-methyl}-pyridinium chloride (**33**)* This compound was prepared from **12** (0.25 g, 0.9 mmol) and nicotinamide (0.5 g, 4 mmol) to yield **33** as white solid (0.32 g, 90%); mp 257–258°C (Decomp.), IR (KBr): $\nu_{max} = 3470, 3351, 1710, 1641, 1538\ cm^{-1}$, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 5.80$ (s, 2H, CH_2), 6.81 (s, 4H, guanido), 7.71 (s, 4H, phenyl), 8.19 (s, 1H, $CONH_2$), 8.31 (dd, 1H, pyridinium, $J = 6.3$ Hz), 8.76, (s, 1H, $CONH_2$), 9.011 (d, 1H, pyridinium, $J = 7.8$ Hz), 9.20 (d, 1H, pyridinium, $J = 5.7$ Hz), 9.59 (s, 1H, pyridinium), 11.50 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 63.13$ (CH_2), 119.54 (2 × CH), 127.93 (2 × CH), 127.91 (CH), 133.90 (C), 140.29 (C), 141.32 (C), 144.83 (CH), 147.45 (CH), 148.75 (CH), 158.86 (C = N), 163.47 (C=O), 164.32 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{15}H_{17}N_6O_4S$: 377.10320, found: 377.10265.

*1-Allylcarbamoylmethyl-3-carbamoyl-pyridinium chloride (**34**)* This compound was prepared from **14** (0.25 g, 1.9 mmol) and nicotinamide (0.5 g, 4 mmol) to yield **34** as pale white solid (0.34 g, 71%); mp 179–180°C (Decomp.), IR (KBr): $\nu_{max} = 3286, 1689, 1550\ cm^{-1}$, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 3.69$ (d, 2H, $J = 4.8$ Hz), 5.03 (dd, 1H, Allylic CH, $J = 3.0$ Hz, 1.5 Hz), 5.21 (dd, 1H, Allylic CH, $J = 1.5$, 3.0 Hz), 5.66 (s, 2H, CH_2), 5.76 (ddd, 1H, Allylic CH, $J = 5.4$ Hz, 10.2 Hz, 12.0 Hz), 8.17 (s, 1H, CO–NH_2), 8.26 (dd, 1H, pyridinium, $J = 6.3$ Hz), 8.97 (s, 1H, CO–NH_2), 9.20 (m, 3H, pyridinium), 9.63 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 42.03$ (CH_2), 62.29 (CH_2), 116.45 (CH_2), 127.85 (CH), 133.69 (C), 134.90 (CH), 144.84 (CH), 147.06 (CH), 148.50 (CH), 163.37 (C=O), 164.75 (C=O); HRMS-FAB *m/z* $[M]^+$ calcd for $C_{11}H_{14}N_3O_2$: 220.10805, found: 220.10806.

*1-(Benzylcarbamoyl-methyl)-pyridinium chloride (**35**)* This compound was prepared from **1** (0.25 g, 1.3 mmol) and pyridine of (1.0 ml, 17 mmol) to yield **35** as pale white solid (0.24 g, 67%); mp 217–218°C (Decomp.), IR (KBr): $\nu_{max} = 3198, 1690, 1648, 1558\ cm^{-1}$, ^1H NMR (300 MHz, MeOH-d_3): $\delta = 4.44$ (s, 2H, CH_2), 5.57 (s, 2H, CH_2), 7.26 (dd, 1H, phenyl, $J = 1.8$ Hz, 3.0 Hz), 7.32 (m, 4H, phenyl), 8.10 (dd, 2H, pyridinium, $J = 6.9$ Hz), 8.61 (dd, 1H, pyridinium, $J = 7.5$ Hz), 8.95 (d, 2H, pyridinium, $J = 6.0$ Hz) ppm; ^{13}C NMR (75 MHz, MeOH-d_3): $\delta =$ 42.69 (CH_2), 61.05 (CH_2), 126.59 (CH), 126.87 (2 × CH), 127.02 (2 × CH), 127.76 (2 × CH), 137.26 (C), 145.50 (2 × CH), 145.61 (CH), 163.87 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{14}H_{15}N_2O$: 227.11844, found: 227.11789; Anal. Calcd for $C_{14}H_{15}N_2OCl$; C: 64.00, H: 5.8, N: 10.7 found: C: 64.06, H: 5.77, N: 10.77.

*1-Phenylcarbamoyl-methyl-pyridinium chloride (**36**)* This compound was prepared from **3** (0.25 g, 1.5 mmol) and pyridine (1.0 ml, 17 mmol) to yield **36** as white powder (0.311 g, 85%); mp 254–255°C (Decomp.), IR (KBr): $\nu_{max} = 3200, 1692, 1604, 1554\ cm^{-1}$, ^1H NMR (300 MHz, MeOH-d_3): $\delta = 5.70$ (s, 2H, CH_2), 7.10 (dd, 1H, phenyl, $J = 7.5$ Hz), 7.30 (dd, 2H, phenyl, $J =$ 7.5 Hz), 7.60 (d, 2H, phenyl, $J = 7.8$ Hz), 8.12 (dd, 2H, pyridinium, $J = 7.5$ Hz), 8.63 (dd, 1H, pyridinium, $J = 7.8$ Hz), 9.10 (d, 2H, pyridinium, $J = 5.4$ Hz) ppm; ^{13}C NMR (75 MHz, MeOH-d_3): $\delta = 62.39$ (CH_2), 120.04 (2 × CH), 124.7 (CH), 127.82 (2 × CH), 128.92 (2 × CH), 137.96 (C), 146.4 (2 × CH), 146.5 (CH), 162.91 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{13}H_{13}N_2O$: 213.10279, found: 213.10224.

*1-[(4-Chloro-phenylcarbamoyl)-methyl]-pyridinium chloride (**37**)* This compound was prepared from **4** (0.25 g, 1.2 mmol) and pyridine (1.0 ml, 17 mmol) to yield **37** as white solid (0.25 g, 72%); mp 234–235°C (Decomp.), IR (KBr): $\nu_{max} = 3250, 1695, 1645, 1595\ cm^{-1}$, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 5.80$ (s, 2H, CH_2), 7.4 (d, 2H, $J = 8.4$ Hz, H2, H6), 7.69 (d, 2H, $J = 8.4$ Hz, H3, H5), 8.18 (dd, 2H, $J = 6.9$ Hz, H2′, H4′), 8.65 (dd, 1H, $J = 7.5$ Hz, H3′), 9.12 (d, 2H, $J = 6.0$ Hz, H1′, H5′), 11.79 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 62.85$ (CH_2), 121.43 (2 × CH), 128.03 (2 × CH), 128.20 (C), 129.44 (2 × CH), 138.13 (C), 146.88 (2 × CH), 147.09 (CH, pyridinium), 164.13 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{13}H_{12}ClN_2O$: 247.06381, found: 247.06327.

*1-(4-Methoxy-phenylcarbamoyl-methyl)-pyridinium chloride (**38**)* This compound was prepared from compound **5** (0.25 g, 1.3 mmol) and pyridine (1.0 ml, 17 mmol) to yield **38** as pale gray solid (0.10 g, 48%); mp 207–208°C (Decomp.), IR (KBr): $\nu_{max} = 3200, 1698, 1650, 1610, 1554\ cm^{-1}$, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 3.70$ (s, 3H, CH_3), 5.75 (s, 2H, CH_2), 6.89 (dd, 2H, phenyl, $J = 9.2$), 7.55 (dd, 2H, phenyl, $J = 8.7$ Hz), 8.19 (dd, 2H, pyridinium, $J = 6.9$), 8.66 (dd, 1H, pyridinium, $J = 7.8$ Hz), 9.17 (d, 2H, pyridinium, $J = 5.7$ Hz), 11.32 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 55.84$ (CH_3, OMe), 62.72 (CH_2), 114.64 (2 × CH), 121.31 (2 × CH), 128.19 (2 × CH), 132.234 (C), 146.82 (2 × CH), 147.06 (CH), 156.19 (C), 163.32 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{14}H_{15}N_2O_2$: 243.11350, found: 243.11280.

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*1-[(4-Nitro-phenylcarbamoyl)-methyl]-pyridinium chloride (**39**)* This compound was prepared from **6** (0.25 g, 1.2 mmol) and pyridine (1.0 ml, 17 mmol) to yield **39** as yellowish solid (0.27 g, 79%); mp 281–282°C (Decomp.), IR (KBr): ν_{max} = 3192, 1725, 1644, 1572, 1501 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): δ = 5.83 (s, 2H, CH_2), 7.91 (d, 2H, pyridinium, J = 8.7 Hz), 8.21 (d, 4H, phenyl, J = 6.9), 8.66 (dd, 1H, pyridinium, J = 7.2 Hz), 9.11 (d, 2H, pyridinium, J = 4.5 Hz), 12.20 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 63.053 (CH_2), 119.75 (2 × CH), 125.74 (2 × CH), 128.23 (2 × CH), 143.35 (C), 145.23 (2 × CH), 147.03 (C), 147.17 (CH), 165.18 (C=O); HRMS-FAB *m/z* $[M]^+$ calcd for $C_{13}H_{12}N_3O_3$: 258.08787, found: 258.08732.

*1-(m-Tolylcarbamoyl-methyl)-pyridinium chloride (**40**)* This compound was prepared from **7** (0.25 g, 1.4 mmol) and pyridine (1.0 ml, 17 mmol) to yield **40** as pale white solid (0.312 g, 87%); mp 224–225°C (Decomp.), IR (KBr): ν_{max} = 3251, 3201, 1692, 1634, 1562 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): δ = 2.20 (s, 3H, CH_3), 5.89 (s, 2H, CH_2), 6.84 (d, 1H, phenyl, J = 7.2), 7.14 (dd, 1H, pyridinium, J = 7.8), 7.46 (d, 1H, phenyl, J = 8.4 Hz), 7.49 (s, 1H, phenyl), 8.20 (dd, 2H, phenyl, J = 7.2), 8.66 (dd, 1H, pyridinium, J = 7.8 Hz), 9.20 (d, 2H, pyridinium, J = 6 Hz), 11.64 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 21.88 (CH_3), 62.86 (CH_2), 117.08 (CH), 120.36 (CH), 125.17(CH), 128.19 (CH), 129.32 (2 × CH), 138.67 (C), 139.11 (C), 146.8 (2 × CH), 147.06 (CH), 163.89 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{14}H_{15}N_2O$: 227.11844, found: 227.11789.

*1-{[(4-Sulfamoyl)-phenylcarbamoyl]-methyl}-pyridinium chloride (**41**)* This compound was prepared from **10** (0.25 g, 1 mmol) and pyridine (1.0 ml, 1.7 mmol) to yield **41** as white solid (0.267 g, 81%); mp 273–274°C (Decomp.), IR (KBr): ν_{max} = 3150, 1701, 1601, 1552 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): δ = 5.87 (s, 2H, CH_2), 7.4 (s, 2H, SO_2NH_2), 7.82 (m, 4H, phenyl), 8.22 (dd, 2H, pyridinium, J = 7.2 Hz), 8.69 (dd, 1H, pyridinium, J = 7.8 Hz), 9.15 (d, 2H, pyridinium, J = 6 Hz), 12.0 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 62.93 (CH_2), 119.49 (2 × CH), 127.52 (2 × CH), 128.23 (2 × CH), 139.60 (C), 142.03 (2 × CH), 146.96 (C), 147.15 (CH), 164.7 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{13}H_{14}N_3O_3S$: 292.07559, found: 292.07504.

*1-{[(4-Acetylsulfamoyl)-phenylcarbamoyl]-methyl}-pyridinium chloride (**42**)* This compound was prepared from **11** (0.25 g, 0.9 mmol) and pyridine (1.0 ml 17 mmol) to yield **42** as white solid (0.22 g, 69%); mp 236–237°C (Decomp.), IR (KBr): ν_{max} = 3600, 3448, 3351, 1701, 1631, 1601, 1552 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): = 1.92 (s, CH_3), 5.88 (s, 2H, CH_2), 7.8 (m, 4H, phenyl), 8.24 (d, 2H, pyridinium, J = 5.7 Hz), 8.70 (br s, 1H, pyridinium), 9.17 (d, 2H, pyridinium, J = 5.1 Hz), 12.0 (sharp s, 1H, CONH), 12.22 (br s, 1H, –SO_2–NH–C=O) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 23.97 (CH_3), 62.95 (CH_2), 119.54 (2 × CH), 128.27 (2 × CH), 129.67 (2 × CH), 134.34 (C), 143.57 (2 × CH), 147.02 (C), 147.17 (CH), 169.64 (C=O), 164.96 (C=O); HRMS-FAB *m/z* $[M]^+$ calcd for $C_{15}H_{16}N_3O_4S$: 334.08615, found: 334.08560.

*1-(p-Tolylcarbamoyl-methyl)-pyridinium chloride (**43**)* This compound was prepared from **9** (0.25 g, 1.4 mmol) and pyridine (1.0 ml, 17 mmol) to yield **43** as pale white solid (0.331 g, 92.5%); mp 253–254°C (Decomp.), IR (KBr): ν_{max} = 3240, 1680, 1601, 1552 cm^{-1}, ^1H NMR (300 MHz, MeOH-d_3): δ = 2.20 (s, 3H, CH_3), 5.71 (s, 2H, CH_2), 7.09 (d, 2H, phenyl, J = 8.1 Hz), 7.46 (d, 2H, phenyl, J = 8.4 Hz), 8.01 (dd, 2H, pyridinium, J = 7.5 Hz), 8.60 (dd, 1H, pyridinium, J = 7.8 Hz), 9.00 (d, 2H, pyridinium, J = 5.4 Hz) ppm; ^{13}C NMR (75 MHz, MeOH-d_3): δ = 19.07 (CH_3), 61.57 (CH_2), 119.31 (2 × CH), 119.41 (2 × CH), 126.99 (2 × CH), 128.56 (2 × CH), 133.66 (C), 134.57 (C), 145.61 (CH), 161.95 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{14}H_{15}N_2O$: 227.11844, found: 227.11789.

*1-{[4-(Guanido-sulfonyl)-phenylcarbamoyl]-methyl}-pyridinium chloride (**44**)* This compound was prepared from **12** (0.25 g, 9 mmol) and pyridine (1.0 ml, 17 mmol) to yield **44** as white solid (0.28 g, 88%); mp 192–194°C (Decomp.), IR (KBr): ν_{max} = 3603, 3448, 3361, 1708, 1634, 1601 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): δ = 5.75 (s, 2H, CH_2), 6.78 (s, 4H, guanido), 7.72 (d, 4H, phenyl, J = 9.0 Hz), 8.21 (dd, 2H, pyridinium, J = 7.2 Hz), 8.68 (dd, 1H, pyridinium, J = 7.8 Hz), 9.08 (d, 2H, pyridinium, J = 5.7 Hz), 11.49 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 62.91 (CH_2), 119.40 (2 × CH), 127.43 (2 × CH), 128.23 (2 × CH), 140.34 (C), 141.31 (C), 146.98 (2 × CH), 147.15 (CH), 158.88 (C = N), 164.47 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{14}H_{16}N_5O_3S$: 334.09738, found: 334.09684.

*3-Phenylcarbamoyl-1-{[(4-sulfamoyl)-phenylcarbamoyl]-methyl}-pyridinium chloride (**45**)* This compound was prepared from **10** (0.25 g, 1 mmol) and **15** (0.5 g, 2.5 mmol) to yield **45** as white solid (0.21 g, 47%); mp 214–215°C (Decomp.), IR (KBr): ν_{max} = 3300, 3248, 1698, 1601, 1552 cm^{-1}, 1H NMR (300 MHz, DMSO-d_6): δ = 5.86 (s, 2H, CH_2), 7.17 (dd, 1H, phenyl, J = 7.2 Hz), 7.38 (m, 4H, phenyl), 7.80 (s, 4H, phenyl), 7.85 (s, 2H,

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NH_2), 8.36 (dd, 1H, pyridinium, $J = 7.2$ Hz), 9.27 (dd, 2H, pyridinium, $J = 6.0$ Hz), 9.77 (s, 1H, pyridinium), 11.31 (s, 1H, CONH), 11.679 (s, 1H, CONH); ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 63.15$ (CH_2), 119.54 (2 × CH), 121.21 (2 × CH), 125.37 (CH), 127.57 (2 × CH), 127.72 (CH), 129.53 (2 × CH), 134.44 (C), 138.94 (C), 139.70 (C), 141.89 (C), 145.48 (CH), 147.54 (CH), 148.86 (CH), 160.99 (C=O), 164.42 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{20}H_{19}N_4O_4S$: 411.11270, found: 411.11215.

*3-Phenylcarbamoyl-1-[(4-nitro-phenylcarbamoyl)-methyl]-pyridinium chloride (**46**)* This compound was prepared from **6** (0.25 g, 1.1 mmol) and **15** (0.5 g, 2.5 mmol) to yield **46** as yellowish solid (0.41 g, 85%); mp 297–298°C (Decomp.), IR (KBr): $\nu_{max} = 3260$, 3201, 1691, 1608, 1562 cm^{-1}, ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 5.93$ (s, 2H, CH_2), 7.14 (dd, 1H, phenyl, $J = 7.2$ Hz), 7.37 (dd, 2H, phenyl, $J = 7.5$ Hz), 7.89 (m, 4H, phenyl), 8.21 (dd, 2H, phenyl, $J = 7.5$ Hz), 8.38 (dd, 1H, pyridinium, $J = 6.6$ Hz), 9.30 (dd, 2H, pyridinium, $J = 8.4$ Hz), 9.82 (s, 1H, pyridinium), 11.36 (s, 1H, CONH), 12.13 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 63.26$ (CH_2), 119.74 (2 × CH), 121.21 (2 × CH), 125.32 (CH), 125.78 (2 × CH), 127.74 (CH), 129.49 (2 × CH), 134.42 (C), 138.97 (C), 143.33 (C), 145.18 (C), 145.58 (CH), 147.61 (CH), 148.87 (CH), 160.99 (C=O), 164.96 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{20}H_{17}N_4O_4$: 377.12498, found: 377.12443.

*3-Phenylcarbamoyl-1-[(4-chloro-phenylcarbamoyl)-methyl]-pyridinium chloride (**47**)* This compound was prepared from **4** (0.25 g, 1.2 mmol) and **15** (0.5 g, 2.5 mmol) to yield **47** as pale solid (0.189 g, 41%); mp 302–303°C (Decomp.), IR (KBr): $\nu_{max} = 3093$, 1681, 1604, 1550 cm^{-1}, ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 5.82$ (s, 2H, CH_2) 7.15 (dd, 2H, phenyl, $J = 7.8$ Hz), 7.38 (m, 4H, phenyl), 7.67 (d, 2H, phenyl, $J = 7.2$ Hz), 7.85 (d, 2H, phenyl, $J = 7.5$ Hz), 8.34 (dd, 1H, pyridinium, $J = 6.6$ Hz, 1.5 Hz), 9.22 (d, 1H, pyridinium, $J = 6.0$ Hz), 9.27 (d, 1H, pyridinium, $J = 8.4$ Hz), 9.78 (s, 1H, pyridinium), 11.32 (s, 1H, CONH), 11.48 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 63.05$ (CH_2), 121.23, (2 × CH), 121.49 (2 × CH), 125.33 (CH), 127.67 (2 × CH), 128.48 (C), 129.48 (2 × CH), 129.54 (CH), 134.44 (C), 138.02 (C), 138.98 (C), 145.47 (CH), 147.47 (CH), 148.82 (CH), 160.95 (C=O), 163.88 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{20}H_{17}N_3O_2Cl$: 366.10038, found: 366.10014.

*3-Phenylcarbamoyl-1-[(4-methoxy-phenylcarbamoyl)-methyl]-pyridinium chloride (**48**)* This compound was prepared from **5** (0.25 g, 1.3 mmol) and **15** (0.5 g, 2.5 mmol) to yield **48** as pale solid (0.271 g, 59%); mp 287–288°C (Decomp.), IR (KBr): $\nu_{max} = 3194$, 1674, 1604, 1550 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.69$ (s, 3H, OCH_3), 5.81 (s, 2H, CH_2), 7.16 (dd, 1H, $J = 7.2$ Hz), 7.38 (dd, 2H, phenyl, $J = 7.2$ Hz), 7.56 (dd, 2H, phenyl, $J = 8.7$ Hz), 7.87 (d, 2H, phenyl, $J = 8.7$ Hz), 7.89 (d, 2H, $J = 8.7$ Hz), 8.35 (dd, 1H, pyridinium, $J = 6.3$ Hz), 9.247 (d, 1H, pyridinium, $J = 5.7$ Hz), 9.31 (d, 1H, pyridinium, $J = 7.8$ Hz), 9.82 (s, 1H, pyridinium), 11.17 (s, 1H, CONH), 11.43 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta =$ 55.84 (s, CH_3, OCH_3), 62.94 (CH_2), 114.69 (2 × CH), 121.24 (2 × CH), 121.38 (2 × CH), 125.31 (CH), 127.67 (CH), 129.49 (2 × CH), 132.18 (C), 134.34 (C), 139.03 (C), 145.40 (CH), 147.45 (CH), 148.82 (CH), 156.27 (C), 160.99 (C=O), 163.08 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{21}H_{20}N_3O_3$: 362.14992, found: 362.14990.

*3-Phenylcarbamoyl-1-[(p-tolylcarbamoyl)-methyl]-pyridinium chloride (**49**)* This compound was prepared from **9** (0.25 g, 1.4 mmol) and **15** (0.5 g, 2.5 mmol) to yield **49** as white solid (0.42 g, 81%); mp 297–298°C (Decomp.), IR (KBr): $\nu_{max} = 3178$, 1682, 1605, 1551 cm^{-1}, ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 2.23$ (s, 1H, CH_3), 5.80 (s, 2H, CH_2), 7.15 (dd, 3H, phenyl, $J = 7.5$ Hz), 7.39 (dd, 2H, phenyl, $J = 7.2$ Hz), 7.50 (d, 2H, phenyl, $J = 8.1$ Hz), 7.85 (d, 2H, phenyl, $J = 8.4$ Hz), 9.22 (d, 1H, pyridinium, $J = 1.8$, 6.6 Hz), 9.28 (d, 1H, pyridinium, $J = 7.8$ Hz), 9.79 (s, 1H, pyridinium), 11.15 (s, 1H, CONH), 11.37 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 21.16$ (CH_3), 63.01 (CH_2), 119.844 (2 × CH), 121.21 (2 × CH), 125.33 (CH), 127.68 (2 × CH), 129.51 (2 × CH), 130.014 (CH), 133.59 (C), 134.40 (C), 136.55 (C), 139.0 (C), 145.40 (CH), 147.45 (CH), 148.82 (CH), 161.0 (C=O), 163.39 (C=O); HRMS-FAB *m/z* $[M]^+$ calcd for $C_{21}H_{20}N_3O_2$: 346.15555, found: 346.15500.

*1-[(4-Methylsulfanyl-phenylcarbamoyl)-methyl]-3-(phenyl carbamoyl)-pyridinium chloride (**50**)* This compound was prepared from **13** (0.25 g, 1.1 mmol) and **15** (0.5 g, 2.5 mmol) to yield **50** as gray solid (0.123 g, 43%); mp 259–260°C (Decomp.), IR (KBr): $\nu_{max} = 3078$, 1681, 1604, 1543 cm^{-1}, ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 2.41$ (s, 3H, CH_3), 5.76 (s, 2H, CH_2), 7.15 (dd, 1H, phenyl, $J = 7.2$ Hz), 7.22 (d, 2H, phenyl, $J = 8.4$ Hz), 7.38 (dd, 2H, phenyl, $J = 7.2$ Hz), 7.56 (d, 2H, phenyl, $J = 7.4$ Hz), 7.80 (d, 2H, phenyl, $J = 7.8$ Hz), 8.34 (dd, 1H, pyridinium, $J = 8.1$ Hz), 9.19 (d, 1H, pyridinium, $J = 7.5$ Hz), 9.22 (d, 1H, pyridinium, $J = 10.2$ Hz), 9.71 (s, 1H, pyridinium), 11.09 (s, 1H, CONH), 11.19 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta =$ 15.91 (CH_3), 63.01 (CH_2), 120.58 (2 × CH), 121.20 (2 × CH), 125.38 (CH), 127.65 (2 × CH), 127.72 (CH), 129.53 (2 × CH), 133.51 (C), 134.51 (C), 136.31 (C), 138.89 (C),

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145.35 (CH), 147.39 (CH), 148.82 (CH), 160.99 (C=O), 163.49 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{21}H_{20}N_3O_2S$: 378.12707, found: 378.12710.

*1-[(4-Chloro-phenylcarbamoyl)-methyl]-3-m-tolylcarbamoyl-pyridinium chloride (**51**)* This compound was prepared from **4** (0.25 g, 1.2 mmol) and **16** (0.5 g, 2.4 mmol) to yield **51** as pale solid (0.10 g, 20%); mp 277–279°C (Decomp.), IR (KBr): $\nu_{max} = 3255, 3209, 1681, 1597, 1550\ cm^{-1}$, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 2.30$ (s, 3H, CH_3), 5.77 (s, 2H, CH_2), 6.98 (d, 1H, phenyl, $J = 6.6$ Hz), 7.26 (br s, 1H, phenyl), 7.38 (d, 2H, phenyl, $J = 7.8$ Hz), 7.61 (br s, 4H, phenyl), 8.35 (br s, 1H, pyridinium), 9.19 (br s, 1H, pyridinium), 9.69 (s, 1H, pyridinium), 11.07 (s, 1H, CONH), 11.28 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 21.86$ (CH_3), 63.046 (CH_2), 118.38 (CH), 121.48 (2 × CH), 121.66 (CH), 126.07 (CH), 127.74 (CH), 128.26 (C), 129.38 (CH), 129.59 (2 × CH), 134.58 (C), 137.94 (C), 138.77 (C), 138.81 (C), 145.36 (CH), 147.39 (CH), 148.82 (CH), 160.90 (C=O), 163.88 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{21}H_{19}N_3O_2Cl$: 380.11658, found: 380.11603.

*1-[(p-Tolylcarbamoyl)-methyl]-3-m-tolylcarbamoyl-pyridinium chloride (**52**)* This compound was prepared from **9** (0.25 g, 1.4 mmol) and **16** (0.5 g, 2.4 mmol) to yield **52** as white solid (0.129 g, 24%); mp 256–258°C (Decomp.), IR (KBr): $\nu_{max} = 3032, 1681, 1604, 1550\ cm^{-1}$, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 2.24$ (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 5.76 (s, 2H, CH_2), 6.90 (d, 1H, $J = 7.8$ Hz), 7.13 (d, 2H, $J = 7.5$ Hz), 7.28 (dd, 1H, $J = 7.5$ Hz), 7.50 (d, 2H, phenyl, $J = 8.4$ Hz), 7.61 (d, 2H, phenyl, $J = 7.8$ Hz), 8.36 (dd, 1H, pyridinium, $J = 7.5$ Hz), 9.21 (d, 2H, pyridinium, $J = 5.1$ Hz), 9.70 (s, 1H, pyridinium), 10.97 (s, 1H, CONH),11.10 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 21.15$ (CH_3), 21.89 (CH_3), 62.99 (CH_2), 118.35 (CH), 119.83 (2 × CH), 121.63 (CH), 126.07 (CH), 127.71 (CH), 129.41 (CH), 130.06 (2 × CH), 133.65 (C), 134.55 (C), 136.48, (C), 138.78 (C), 138.84 (C), 145.30 (CH), 147.37 (CH), 148.84 (CH), 160.95 (C=O), 163.40 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{22}H_{22}N_3O_2$: 360.17065, found: 360.17060.

*1-[(4-Chloro-phenylcarbamoyl)-methyl]-3-allyl-carbamoyl-pyridinium chloride (**53**)* This compound was prepared from **4** (0.25 g, 1.2 mmol) and **21** (0.5 g, 3 mmol) to yield **53** as pale solid (0.319 g, 71%); mp 228–230°C (Decomp.), IR (KBr): $\nu_{max} = 3255, 3225. 1666, 1604, 1551\ cm^{-1}$, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 3.91$ (br s, 2H, allylic CH_2), 5.07 (dd, 1H, allyl, $J = 1.2, 10.2$ Hz), 5.22 (s, 2H, CH_2), 5.19 (dd, 1H, allyl, $J = 1.2, 17.1$ Hz), 5.91 (ddd, 1H, allyl CH, $J = 3.6, 5.1, 10.2$ Hz), 7.67 (d, 2H, phenyl, $J = 8.7$ Hz), 7.34 (d, 2H, phenyl, $J = 8.7$ Hz), 8.31 (dd, 1H, $J = 0.9, 7.2$ Hz), 9.17 (d, 1H, pyridinium, $J = 8.1$ Hz), 9.23 (d, 1H, pyridinium, $J = 5.4$ Hz), 9.63 (s, 1H, pyridinium), 11.68 (s, 1H, CO–NH), 9.72 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 42.45$ (CH_2), 63.01 (CH_2), 116.60 (CH_2), 121.39 (2 × CH), 127.85 (2 × CH), 128.09 (C), 129.47 (CH), 133.77 (C), 134.96 (CH), 138.07 (C), 144.734 (CH), 147.174 (CH), 148.69 (CH), 161.68 (C=O), 163.92 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{17}H_{17}N_3O_2Cl$: 330.10038, found: 330.10064.

*1-[(4-Methoxy-phenylcarbamoyl)-methyl]-3-allyl-carbamoyl-pyridinium chloride (**54**)* This compound was prepared from **5** (0.25 g, 1.3 mmol) and **21** (0.5 g, 3 mmol) to yield **54** as grayish pale solid (0.388 g, 86%); mp 211–212°C (Decomp.), IR (KBr): $\nu_{max} = 3217, 3178, 1666, 1597, 1551\ cm^{-1}$, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 3.70$ (s, 3H, CH_3), 3.97 (s, 2H, allyl CH_2), 5.10 (d, 1H, allyl, $J = 0.9$ Hz), 5.23 (d, 1H, allyl, $J = 16.5$ Hz), 5.75 (s, 2H, CH_2), 5.90 (m, 1H, CH, allyl, $J = 4.8$ Hz), 7.54 (d, 2H, phenyl, $J = 9.0$ Hz), 7.89 (d, 2H, phenyl, $J = 8.7$ Hz), 8.32 (dd, 1H, 6.6 Hz), 9.12 (d, 1H, pyridinium, $J = 8.1$ Hz), 9.19 (d, 1H, pyridinium, $J = 5.1$ Hz), 9.59 (s, 1H, pyridinium), 9.64 (s, 1H, CONH), 11.09 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 42.48$ (CH_2), 55.84 (CH_3), 62.96 (CH_2), 114.69 (CH), 116.64 (CH2), 121.34 (2 × CH), 127.86 (2 × CH), 132.14 (C), 133.83 (C), 134.99 (CH), 144.62 (CH), 147.11 (CH), 148.70 (CH), 156.26 (C), 161.77 (C=O), 163.10 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{18}H_{20}N_3O_3$: 326.14992, found: 326.14988.

*1-[(4-Nitro-phenylcarbamoyl)-methyl]-3-allyl-carbamoyl-pyridinium chloride (**55**)* This compound was prepared from **6** (0.25 g, 1.1 mmol) and **21** (0.5 g, 3 mmol) to yield **55** as yellowish pale solid (0.22 g, 50%); mp 241–242°C (Decomp.), IR (KBr): $\nu_{max} = 3201, 1681, 1566\ cm^{-1}$, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 3.93$ (s, 2H, allyl), 5.09 (d, 1H, allyl, $J = 10.2$ Hz), 5.20 (d, 1H, allyl, $J = 17.4$ Hz), 5.85 (m, 1H, allyl), 5.88 (s, 2H, CH_2), 7.90 (d, 2H, phenyl, $J = 9.0$ Hz), 8.21 (d, 2H, phenyl, $J = 8.1$ Hz), 8.32 (dd, 1H, $J = 6.6$ Hz), 9.15 (d, 1H, pyridinium, $J = 8.4$ Hz), 9.23 (d, 1H, pyridinium, $J = 6.0$ Hz), 9.62 (s, 1H, pyridinium), 9.65 (s, 1H, CONH), 12.19 (br s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 42.45$ (CH_2), 63.26 (CH_2), 116.60 (CH_2), 119.72 (2 × CH), 125.76 (2 × CH), 127.90 (CH), 133.85 (C), 134.96 (CH), 143.30 (C), 144.81 (CH), 145.21 (C), 147.28 (CH), 148.74 (CH), 161.72 (C=O), 164.99 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{17}H_{17}N_4O_4$: 341.12498, found: 341.12443.

*1-[(p-Tolylcarbamoyl)-methyl]-3-allyl-carbamoyl-pyridinium chloride (**56**)* This compound was prepared from **9**

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(0.25 g, 1.4 mmol) and **21** (0.5 g, 3 mmol) to yield **56** as white solid (0.32 g, 68%); mp 219–220°C (Decomp.), IR (KBr): $\nu_{max} = 3240, 3178, 1666, 1612, 1550\ cm^{-1}$, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 3.92$ (br s, 2H, H4″), 5.09 (ddd, 1H, allyl, $J = 1.5, 3.0, 11.7$ Hz, H2″), 5.24 (ddd, 1H, allyl, $J = 1.5, 3.0, 16.8$ Hz, H1″), 5.77 (s, 2H, CH_2), 5.91 (ddd, 1H, $J = 5.1, 10.5, 15.6$ Hz, H3″), 7.10 (d, 2H, $J = 8.1$ Hz, H3,H5), 7.50 (d, 2H, $J = 8.1$ Hz, H2,H6), 8.30 (dd, 1H, $J = 7.5$ Hz, H4′), 9.13 (d, 1H, $J = 8.1$ Hz, H5′), 9.19 (d, 1H, $J = 6.0$ Hz, H3′), 9.60 (s, 1H, H1′), 9.68 (s, 1H, CO–NH), 11.21 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 21.14$ (CH_3), 42.45 (CH_2), 63.01 (CH_2), 116.6 (CH_2), 119.8 (2 × CH), 127.84 (2 × CH), 129.96 (CH), 133.52 (C), 133.8 (C), 134.98 (CH), 136.56 (C), 144.65 (CH), 147.12 (CH), 148.68 (CH), 161.73 (C=O), 163.4 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{18}H_{20}N_3O_2$: 310.15555, found: 310.15500.

*3-(4-Methoxy-phenylcarbamoyl)-1-[(4-methoxy-phenyl carbamoyl)-methyl]-pyridinium chloride (**57**)* This compound was prepared from **5** (0.25 g, 1.3 mmol) and **18** (0.5 g, 2 mmol) to yield **57** as grayish white solid (0.142 g, 30%); mp 241–242°C (Decomp.), IR (KBr): $\nu_{max} = 3309, 3109, 1666, 1512\ cm^{-1}$, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 3.72$ (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 5.80 (s, 2H, CH_2), 6.92 (2H, phenyl, $J = 8.4$ Hz), 6.98 (d, 2H, phenyl, $J = 7.2$ Hz), 7.57 (d, 2H, phenyl, $J = 6.9$ Hz), 7.78 (d, 2H, phenyl, $J = 7.5$ Hz), 8.36 (dd, 1H, pyridinium, $J = 6.6$ Hz), 9.24 (d, 1H, pyridinium, $J = 8.4$ Hz), 9.30 (d, 1H, pyridinium, $J = 7.5$ Hz), 9.78 (s, 1H, pyridinium), 11.12 (s, 1H, CONH), 11.27 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 55.86$ (CH_3, OCH_3), 55.92 (CH_3, OCH_3), 62.94 (CH_2), 114.62 (2 × CH), 114.72 (2 × CH), 121.38 (2 × CH), 122.78 (2 × CH), 127.7 (CH), 132.04 (C), 132.17 (C), 134.48 (C), 145.24 (CH), 147.34 (CH), 148.7 (CH), 156.28 (C), 156.84 (C), 160.49 (C=O), 163.11 (C=O); HRMS-FAB *m/z* $[M]^+$ calcd for $C_{22}H_{22}N_3O_4$: 392.16048, found: 392.16016.

*3-(4-Methoxy-phenylcarbamoyl)-1-[(4-nitro-phenylcarbamoyl)-methyl]-pyridinium chloride (**58**)* This compound was prepared from **6** (0.25 g, 1.1 mmol) and **18** (0.5 g, 2 mmol) to yield **58** as yellowish pale solid (0.376 g, 77.5%); mp 270–271°C (decomp.), IR (KBr): $\nu_{max} = 3271, 3201, 1674, 1612, 1512\ cm^{-1}$, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 3.77$ (s, 3H, OCH_3), 5.95 (s,2H, CH_2), 6.98 (d, 2H, phenyl, $J = 8.7$ Hz), 7.78 (d, 2H, phenyl, $J = 8.7$ Hz), 7.94 (d, 2H, phenyl, $J = 9.0$ Hz), 8.26 (d, 2H, phenyl, $J = 9.0$), 8.35 (dd, 1H, pyridinium, $J = 6.6$ Hz), 9.28 (d, 1H, pyridinium, $J = 6.0$ Hz), 9.33 (d, 1H, pyridinium, $J = 8.1$ Hz), 9.82 (s, 1H, pyridinium), 11.23 (s, 1H, CONH), 12.10 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 55.15$ (CH_3, OCH_3), 62.49 (CH_2), 113.85 (2 × CH), 119.03 (2 × CH), 122.03 (2 × CH), 124.99 (CH), 126.99 (2 × CH), 131.20 (C), 133.8 (C), 142.63 (C), 144.38 (C), 144.62 (CH), 146.71 (CH), 147.96 (CH), 156.12 (C), 159.65 (C=O), 164.17 (C=O); HRMS-FAB *m/z* $[M]^+$ calcd for $C_{21}H_{19}N_4O_5$: 407.13500, found: 407.13501.

*3-(4-Methoxy-phenylcarbamoyl)-1-[(p-tolylcarbamoyl)-methyl]-pyridinium chloride (**59**)* This compound was prepared from **9** (0.25 g, 1.4 mmol) and **18** (0.5 g, 2 mmol) to yield **59** as grayish pale solid (0.154 g, 34%); mp 251–252°C (decomp.), IR (KBr): $\nu_{max} = 3333, 3256, 1674, 1604, 1512\ cm^{-1}$, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 2.23$ (s, 3H, CH_3), 3.73 (s, 3H, OCH_3), 5.79 (s, 2H, CH_2), 6.94 (2H, phenyl, $J = 8.4$ Hz), 7.10 (d, 2H, phenyl, $J = 7.8$ Hz), 7.51 (d, 2H, phenyl, $J = 7.8$ Hz), 7.75 (d, 2H, phenyl, $J = 9.0$ Hz), 8.32 (dd, 1H, pyridinium, $J = 6.9$ Hz), 9.21 (d, 1H, pyridinium, $J = 5.1$ Hz), 9.27 (d, 1H, pyridinium, $J = 7.8$ Hz), 9.76 (s, 1H, pyridinium), 11.11 (s, 1H, CONH), 11.21 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 21.13$ (CH_3), 55.92 (CH_3, OCH_3), 63.02 (CH_2), 114.61 (2 × CH), 119.9 (2 × CH), 122.797 (2 × CH), 127.68 (CH), 129.977 (2 × CH), 132.00 (C), 133.62 (C), 134.5 (C), 136.51 (C), 145.23 (CH), 147.31 (CH), 148.65 (CH), 156.87, (C), 160.44 (C=O), 163.36 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{22}H_{22}N_3O_3$: 376.16612, found: 376.16557.

*3-(4-Methoxy-phenylcarbamoyl)-1-[(4-chloro-phenylcarbamoyl)-methyl]-pyridinium chloride (**60**)* This compound was prepared from **4** (0.25 g, 1.2 mmol) and **18** (0.5 g, 2 mmol) to yield **60** as pale white solid (0.347 g, 73%); mp 238–239°C (decomp.), IR (KBr): $\nu_{max} = 3340, 1604, 1512\ cm^{-1}$, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 3.76$ (s, 3H, OCH_3), 5.83 (s, 2H, CH_2), 6.98 (2H, phenyl, $J = 8.4$ Hz), 7.41 (d, 2H, phenyl, $J = 8.4$ Hz), 7.69 (d, 2H, phenyl, $J = 8.4$ Hz), 7.76 (d, 2H, phenyl, $J = 8.4$ Hz), 8.37 (dd, 1H, pyridinium, $J = 6.6$ Hz), 9.24 (d, 1H, pyridinium, $J = 8.4$ Hz), 9.28 (d, 1H, pyridinium, $J = 7.8$ Hz), 9.77 (s, 1H, pyridinium), 11.18 (s, 1H, CONH), 11.43 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 55.15$ (CH_3, OCH_3), 62.27 (CH_2), 113.86 (2 × CH), 120.72 (2 × CH), 122.01 (2 × CH), 126.95 (C), 127.46 (CH), 128.78 (2 × CH), 131.18 (C), 133.78 (C), 137.21 (C), 144.49 (CH), 146.58 (CH), 147.92 (CH), 156.11 (C), 159.66 (C=O), 163.11 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{21}H_{19}N_3O_3Cl$: 396.11149, found: 396.11095.

*3-(4-Methoxy-phenylcarbamoyl)-1-[(4-Sulfamoyl-phenyl carbamoyl)-methyl]-pyridinium chloride (**61**)* This compound is prepared from **10** (0.25 g, 1 mmol) and **18** (0.5 g, 2 mmol) to yield **61** as pale white solid (0.10 g, 19%); mp

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250–252°C (decomp.), IR (KBr): $\nu_{max} = 3317$, 3232, 3186, 1674, 1597, 1550 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 3.74$ (s, 3H, OCH_3), 5.81 (s, 2H, CH_2), 6.96 (d, 2H, phenyl, $J = 9.0$ Hz), 7.28 (s, 2H, NH_2), 7.70 (d, 2H, phenyl, $J = 9.0$ Hz), 7.77 (s, 4H, phenyl), 8.35 (dd, 1H, pyridinium, $J = 1.5$, 6.6 Hz), 9.20 (dd, 2H, pyridinium, $J = 9.6$ Hz), 9.69 (s, 1H, pyridinium), 11.029 (s, 1H, CONH), 11.46 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 55.92$ (CH_3, OCH_3), 63.13 (CH_2), 114.68 (2 × CH), 119.57 (2 × CH), 122.78 (2 × CH), 127.57 (2 × CH), 127.74 (CH), 131.88 (C), 134.63 (C), 139.80 (C), 141.8 (C), 145.23 (CH), 147.41 (CH), 148.73 (CH), 156.91 (C), 160.47 (C=O), 164.39 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{21}H_{21}N_4O_5S$: 441.12372, found: 441.12272.

*3-(4-Nitro-phenylcarbamoyl)-1-[(4-methoxy-phenylcarbamoyl)-methyl]-pyridinium chloride (**62**)* This compound was prepared from **5** (0.25 g, 1.3 mmol) and **19** (0.5 g, 2 mmol) to yield **62** as yellowish pale solid (0.183 g, 40%); mp 275–276°C (decomp.), IR (KBr): $\nu_{max} = 3039$, 1681, 1612, 1566 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 3.71$ (s, 3H, OCH_3), 5.75 (s, 2H, CH_2), 7.52 (d, 2H, $J = 8.7$ Hz, H3,H5), 7.90 (d, 2H, $J = 8.7$ Hz, H2,H6), 8.13 (d, 2H, $J = 9.3$ Hz, H2″,H6″), 8.30 (d, 2H, $J = 8.7$ Hz, H3″,H5″), 8.37 (dd, 1H, $J = 7.5$ Hz, H4′), 9.21 (d, 1H, $J = 6.0$ Hz, H5′), 9.28 (d, 1H, $J = 8.4$ Hz, H3′), 9.77 (s, 1H, H1′), 10.85 (s, 1H, CONH), 11.85 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 55.86$ (CH_3, OCH_3), 62.96 (CH_2), 114.75 (2 × CH), 120.99 (2 × CH), 121.43 (2 × CH), 125.57 (CH), 127.7 (2 × CH), 132.01 (C), 133.93 (C), 143.92 (C), 145.13 (C), 145.64 (CH), 147.59 (CH), 149.20 (CH), 156.35 (C), 161.90 (C=O), 162.99 (C=O), ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{21}H_{19}N_4O_5$: 407.13500, found: 407.13428.

*3-(4-Nitro-phenylcarbamoyl)-1-[(-p-tolylcarbamoyl)-methyl]-pyridinium chloride (**63**)* This compound was prepared from **9** (0.25 g, 1.4 mmol) and **19** (0.5 g, 2 mmol) to yield **63** as yellowish pale solid (0.127 g, 22%); mp 290–291°C (decomp.), IR (KBr): $\nu_{max} = 3171$, 1681, 1604, 1550 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 2.22$ (s, 3H, CH_3), 5.76 (s, 2H, CH_2), 7.11 (d, 2H, phenyl, $J = 8.4$ Hz), 7.49 (d, 2H, phenyl, $J = 8.1$ Hz), 8.14 (d, 2H, phenyl, $J = 8.7$ Hz), 8.29 (d, 2H, phenyl, $J = 9.0$ Hz), 8.36 (dd, 1H, pyridinium, $J = 7.8$ Hz), 9.19 (d, 1H, pyridinium, $J = 5.7$ Hz), 9.30 (d, 1H, pyridinium, $J = 8.1$ Hz), 9.79 (s, 1H, pyridinium), 10.99 (s, 1H, CONH), 11.95 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 21.12$ (CH_3), 63.03 (CH_2), 119.87 (2 × CH), 121.0 (2 × CH), 125.55 (2 × CH), 127.68 (CH), 130.01 (2 × CH), 133.65 (C), 133.87 (C), 136.46 (C), 143.89 (C), 145.18 (C), 145.71 (CH), 147.65 (CH), 149.2 (CH), 161.90 (C=O), 163.3 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{21}H_{19}N_4O_4$: 391.14008, found: 391.14000.

*3-(4-Nitro-phenylcarbamoyl)-1-[(4-sulfamoyl-phenylcarbamoyl)-methyl]-pyridinium chloride (**64**)* This compound was prepared from **10** (0.25 g, 1 mmol) and **19** (0.5 g, 2 mmol) to yield **64** as yellow pale solid (0.102 g, 20%); mp 281–282°C, IR (KBr): $\nu_{max} = 3155$, 1697, 1558 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 5.83$ (s, 2H, CH_2), 7.28 (s, 2H, NH_2), 7.78 (s, 4H, phenyl), 8.14 (d, 2H, phenyl, $J = 8.7$ Hz), 8.28 (d, 2H, phenyl, $J = 9.3$ Hz), 8.36 (dd, 1H, pyridinium, $J = 6.3$ Hz), 9.22 (br s, 1H, pyridinium), 9.30 (d, 1H, pyridinium, $J = 6.9$ Hz), 9.78 (s, 1H, pyridinium), 11.87 (s, 1H, CONH), 11.50 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 63.15$ (CH_2), 119.56 (2 × CH), 120.75 (2 × CH), 121.01 (2 × CH), 125.57 (CH), 127.57 (2 × CH), 133.95 (C), 139.81 (C), 141.79 (C), 143.94 (C), 145.12 (C), 145.77 (CH), 147.73 (CH), 149.23 (CH), 161.89 (C=O), 164.32 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{20}H_{18}N_5O_6S$: 456.09723, found: 456.09654.

*3-(4-Chloro-phenylcarbamoyl)-1-[(4-methoxy-phenylcarbamoyl)-methyl]-pyridinium chloride (**65**)* This compound was prepared from **5** (0.25 g, 1.3 mmol) and **20** (0.5 g, 2 mmol) to yield **65** as pale white solid (0.237 g, 42%); mp 251–253°C (decomp.), IR (KBr): $\nu_{max} = 3255$, 3178, 1681, 1604, 1550 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 3.73$ (s, 3H, CH_3), 5.78 (s, 2H, CH_2), 6.92 (d, 2H, phenyl, $J = 9.0$ Hz), 7.48 (d, 2H, phenyl, $J = 8.7$ Hz), 7.55 (d, 2H, phenyl, $J = 9.3$ Hz), 7.90 (d, 2H, phenyl, $J = 9.0$ Hz), 8.37 (dd, 1H, pyridinium, $J = 1.8$, 6.6 Hz), 9.23 (d, 1H, pyridinium, $J = 6.0$ Hz), 9.29 (d, 1H, pyridinium, $J = 8.1$ Hz), 9.78 (s, 1H, pyridinium), 10.97 (s, 1H, CONH), 11.48 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 55.08$ (CH_3, OCH_3), 62.16 (CH_2), 113.96 (2 × CH), 120.65 (2 × CH), 121.99 (2 × CH), 126.92 (C), 128.24 (2 × CH), 128.67 (CH), 131.28 (C), 133.46 (C), 137.17 (C), 144.62, (CH), 146.63 (CH), 148.12 (CH), 155.57 (C), 160.34 (C=O), 162.25 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{21}H_{19}N_3O_3Cl$: 396.11095, found: 396.11095.

*3-(4-Chloro-phenylcarbamoyl)-1-[(4-nitro-phenylcarbamoyl)-methyl]-pyridinium chloride (**66**)* This compound was prepared from **6** (0.25 g, 1.1 mmol) and **20** (0.5 g, 2 mmol) to yield **66** as yellowish pale solid (0.16 g, 31%); mp 292–294°C (decomp.), IR (KBr): $\nu_{max} = 3263$, 3194, 1681, 1604, 1504 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 5.90$ (s, 2H, CH_2), 7.48 (d, 2H, phenyl, $J = 8.7$ Hz), 7.89 (d, 2H, phenyl, $J = 3.6$ Hz), 7.92 (d, 2H, phenyl, $J = 3.9$ Hz), 8.27 (d, 2H, phenyl, $J = 9.3$ Hz), 8.40 (dd, 1H, pyridinium, $J = 6.6$ Hz), 9.25 (d, 1H, pyridinium,

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$J = 6.3$ Hz), 9.31 (d, 1H, pyridinium, $J = 8.1$ Hz), 9.78 (s, 1H, pyridinium), 11.45 (s, 1H, CONH), 11.92 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 62.48$ (CH_2), 119.03 (2 × CH), 121.99 (2 × CH), 125.03 (2 × CH), 127.0 (C), 128.27 (2 × CH), 128.7 (CH), 133.53 (C), 137.15 (C), 142.67 (C), 144.3 (C), 144.78 (CH), 146.82 (CH), 148.2 (CH), 160.32 (C=O), 164.12 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{20}H_{16}N_4O_4Cl$: 411.08546, found: 411.08539.

*3-(4-Chloro-phenylcarbamoyl)-1-[(p-tolylcarbamoyl)-methyl]-pyridinium chloride (**67**)* This compound was prepared from **9** (0.25 g, 1.4 mmol) and **20** (0.5 g, 2 mmol) to yield compound **67** as pale white solid (0.10 g, 22%); mp 261–262°C (decomp.), IR (KBr): $\nu_{max} = 3232$, 3178, 1681, 1604, 1550 cm^{-1}, ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 2.27$ (s, 3H, CH_3), 5.78 (s, 2H, CH_2), 7.15 (d, 2H, phenyl, $J = 8.1$ Hz), 7.37 (d, 2H, phenyl, $J = 9.0$ Hz), 7.51 (dd, 4H, phenyl, $J = 2.7$, 9.0 Hz), 7.89 (d, 2H, phenyl, $J = 9.0$ Hz), 8.39 (dd, 1H, pyridinium, $J = 3.3$, 5.4 Hz), 9.25 (dd, 2H, pyridinium, $J = 3.3$, 7.8 Hz), 9.76 (s, 1H, pyridinium), 11.36 (s, 1H, CONH), 11.41 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 20.35$ (CH_3), 62.24 (CH_2), 119.11 (2 × CH), 121.99 (2 × CH), 126.94 (2 × CH), 128.27 (C), 128.71 (2 × CH), 129.25 (CH), 132.91 (C), 133.52 (C), 135.66 (C), 137.15 (C), 144.61 (CH), 146.66 (CH), 148.17 (CH), 160.36 (C=O), 162.56 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{21}H_{19}N_3O_2Cl_1$: 380.11658, found: 380.11603.

*3-(4-Chloro-phenylcarbamoyl)-1-[(4-chloro-phenylcarbamoyl)-methyl]-pyridinium chloride (**68**)* This compound was prepared from **4** (0.25 g, 1.2 mmol) and **20** (0.5 g, 2 mmol) to yield **68** as pale solid (0.158 g, 34%); mp 248–250°C (decomp.), IR (KBr): $\nu_{max} = 3240$, 3178, 1681, 1604, 1543 cm^{-1}, ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 5.79$ (s, 2H, CH_2), 7.37 (d, 2H, phenyl, $J = 9.0$ Hz), 7.44 (d, 2H, phenyl, $J = 9.0$ Hz), 7.65 (d, 2H, phenyl, $J = 8.7$ Hz), 7.88 (d, 2H, phenyl, $J = 8.7$ Hz), 8.35 (dd, 1H, pyridinium, $J = 1.5$, 6.3 Hz), 9.21 (d, 1H, pyridinium, $J = 6.0$ Hz), 9.75 (s, 1H, pyridinium), 11.36 (s, 1H, CONH), 11.46 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 63.05$ (CH_2), 121.49 (2 × CH), 122.77 (2 × CH), 127.72 (2 × CH), 128.25 (C), 129.02 (C), 129.44 (2 × CH), 129.56 (CH), 134.24 (C), 137.90 (C), 137.96 (C), 145.48 (CH), 147.5 (CH), 148.93 (CH), 161.1 (C=O), 163.86 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{20}H_{16}N_3O_2Cl_3$: 400.06141, found: 400.06143.

*3-(4-Chloro-phenylcarbamoyl)-1-[(4-sulfamoyl-phenyl carbamoyl)-methyl]-pyridinium chloride (**69**)* This compound was prepared from **10** (0.25 g, 1 mmol) and **20** (0.5 g, 2 mmol) to yield **69** as white solid (0.16 g, 31%); mp 284–285°C (decomp.), IR (KBr): $\nu_{max} = 3379$, 3271, 3178, 1681, 1604, 1550 cm^{-1}, ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 5.86$ (s, 2H, CH_2), 7.31 (s, 2H, NH_2), 7.48 (d, 2H, phenyl, $J = 8.7$ Hz), 7.81 (s, 4H, phenyl), 7.92 (d, 2H, phenyl, $J = 8.7$ Hz), 8.39 (dd, 1H, pyridinium, $J = 7.8$ Hz), 9.25 (d, 1H, pyridinium, $J = 6.3$ Hz), 9.31 (d, 1H, pyridinium, $J = 7.2$ Hz), 9.79 (s, 1H, pyridinium), 11.47 (s, 1H, CONH), 11.58 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 63.16$ (CH_2), 119.6 (2 × CH), 122.79 (2 × CH), 127.58 (2 × CH), 127.75 (2 × CH), 129.05 (C), 129.49 (CH), 134.3 (C), 137.98, (C), 139.82 (C), 141.85 (C), 145.53 (CH), 147.58 (CH), 148.97 (CH), 161.14 (C=O), 164.39 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{20}H_{18}N_4O_4S_1Cl$: 445.07318, found: 445.07272.

*3-(4-Chloro-phenylcarbamoyl)-1-[(4-methylsulfanyl-phenyl carbamoyl)-methyl]-pyridinium chloride (**70**)* This compound was prepared from **13** (0.25 g, 1.1 mmol) and **20** (0.5 g, 2 mmol) to yield **70** as pale white solid (0.15 g, 48%); mp 235–237°C (decomp.), IR (KBr): $\nu_{max} = 3232$, 3171, 1681, 1604 cm^{-1}, ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 2.39$ (s, 3H, CH_3), 5.73 (s, 2H, CH_2), 7.20 (d, 2H, phenyl, $J = 8.4$ Hz), 7.43 (d, 2H, phenyl, $J = 8.4$ Hz), 7.54 (d, 2H, phenyl, $J = 8.1$ Hz), 7.84 (d, 2H, phenyl, $J = 8.4$ Hz), 8.33 (dd, 1H, pyridinium, $J = 5.7$ Hz), 9.20 (dd, 2H, pyridinium, $J = 7.5$ Hz), 9.70 (s, 1H, pyridinium), 11.05 (s, 1H, CONH), 11.37 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 15.89$ (CH_3), 63.0 (CH_2), 120.56 (2 × CH), 122.74 (2 × CH), 127.63 (2 × CH), 127.71 (C), 129.02 (C), 129.46 (CH), 133.50 (C), 134.26 (2 × CH), 136.28 (C), 137.9 (C), 145.39 (CH), 147.42 (CH), 148.92 (CH), 161.11 (C=O), 163.45 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{21}H_{19}ClN_3O_2S$: 412.08865, found: 412.08810.

*1-[(4-Methoxy-phenylcarbamoyl)-methyl]-3-(4-methylsulfanyl-phenyl-carbamoyl)-pyridinium chloride (**71**)* This compound was prepared from compound **5** (0.25 g, 1.3 mmol) and **17** (0.5 g, 2 mmol) to yield **71** as pale white solid (0.193 g, 34.7%); mp 248–250°C (decomp.), IR (KBr): $\nu_{max} = 3232$, 3171, 1681, 1597 cm^{-1}, ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 2.44$ (s, 3H, CH_3), 3.68 (s, 3H, OCH_3), 5.76 (s, 2H, CH_2), 6.87 (d, 2H, phenyl, $J = 9.0$ Hz), 7.27 (d, 2H, phenyl, $J = 8.1$ Hz), 7.52 (d, 2H, phenyl, $J = 8.4$ Hz), 7.80 (d, 2H, phenyl, $J = 8.1$ Hz), 8.32 (dd, 1H, pyridinium, $J = 6.9$ Hz), 9.20 (d, 1H, pyridinium, $J = 5.7$ Hz), 9.27 (d, 1H, pyridinium, $J = 7.8$ Hz), 9.76 (s, 1H, pyridinium), 11.05 (s, 1H, CONH), 11.36 (s, 1H, CONH) ppm; ^{13}C NMR (300 MHz, DMSO-d_6): $\delta = 15.88$ (CH_3), 55.84 (CH_3, OCH_3), 62.91 (CH_2), 114.69 (2 × CH), 121.42 (2 × CH), 121.83 (2 × CH), 127.35 (2 × CH), 127.66 (CH), 132.10 (C), 134.31 (C),

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134.37 (C), 136.29 (C), 145.32 (CH), 147.34 (CH), 148.73 (CH), 156.30 (C), 160.77 (C=O), 163.02 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{22}H_{22}N_3O_3S$: 408.13764, found: 408.13766.

*3-(4-Methylsulfanyl-phenylcarbamoyl)-1-[(4-nitro-phenyl carbamoyl)-methyl]-pyridinium chloride (**72**)* This compound was prepared from **6** (0.25 g, 1.1 mmol) and **17** (0.5 g, 2 mmol) to yield **72** as a yellowish pale solid (0.234 g, 43.7%); mp 238–239°C (decomp.), IR (KBr): $\nu_{max} = 3217$, 3155, 1674, 1504 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 2.45$ (s, 3H, CH_3), 5.89 (s, 2H, CH_2), 7.28 (d, 2H, phenyl, $J = 8.4$ Hz), 7.79 (d, 2H, phenyl, $J = 8.7$ Hz), 7.89 (d, 2H, phenyl, $J = 9.0$ Hz), 8.21 (d, 2H, phenyl, $J = 9.3$ Hz), 8.36 (dd, 1H, pyridinium, $J = 6.6$, 7.8 Hz), 9.23 (d, 1H, pyridinium, $J = 6.3$ Hz), 9.28 (d, 1H, pyridinium, $J = 8.4$ Hz), 9.77 (s, 1H, pyridinium), 11.30 (s, 1H, CONH), 12.00 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 15.84$ (CH_3), 63.24 (CH_2), 119.76 (2 × CH), 121.71 (2 × CH), 125.65 (2 × CH), 127.34 (2 × CH), 127.74 (CH), 130.31 (CH), 134.43 (C), 136.24 (C), 137.54 (C), 143.38 (C), 145.12 (C), 147.56 (CH), 148.84 (CH), 160.77 (C=O), 164.93 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{21}H_{19}N_4O_4S$: 423.11270, found: 423.11215.

*3-(4-Methylsulfanyl-phenylcarbamoyl)-1-[(p-tolylcarbamoyl)-methyl]-pyridinium chloride (**73**)* This compound was prepared from **9** (0.25 g, 1.4 mmol) and **17** (0.5 g, 2 mmol) to yield **73** as grayish pale solid (0.20 g, 34%); mp 244–246°C (decomp.), IR (KBr): $\nu_{max} = 3232$, 2862, 1674 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 2.22$ (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 5.76 (s, 2H, CH_2), 7.10 (d, 2H, phenyl, $J = 8.1$ Hz), 7.28 (d, 2H, phenyl, $J = 8.7$ Hz), 7.49 (d, 2H, phenyl, $J = 8.4$ Hz), 7.79 (d, 2H, phenyl, $J = 8.7$ Hz), 8.3 (dd, 1H, pyridinium, $J = 6.3$, 7.5 Hz), 9.2 (d, 1H, pyridinium, $J = 6.0$ Hz), 9.25 (d, 1H, pyridinium, $J = 8.1$ Hz), 9.7 (s, 1H, pyridinium), 11.0 (s, 1H, CONH), 11.3 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 15.87$ (CH_3), 21.12 (CH_3), 63.0 (CH_2), 119.89 (2 × CH), 121.68 (2 × CH), 127.38 (2 × CH), 127.69 (2 × CH), 129.99 (CH), 133.64 (C), 134.36 (C), 134.42 (C), 136.26 (C), 136.47 (C), 145.31 (CH), 147.36 (CH), 148.76 (CH), 160.79 (C=O), 163.33 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{22}H_{22}N_3O_2S$: 392.14327, found: 392.143.

*1-[(4-Chloro-phenylcarbamoyl)-methyl]-3-(4-methylsulfanyl-phenyl-carbamoyl)-pyridinium chloride (**74**)* This compound was prepared from **4** (0.25 g, 1.2 mmol) and **17** (0.5 g, 2 mmol) to yield **74** as pale white solid (0.165 g, 30%): mp 228–229°C (decomp.), IR (KBr): $\nu_{max} = 3232$, 3171, 1674, 1604, 1543 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 2.45$ (s, 3H, CH_3), 5.76 (s, 2H, CH_2), 7.29 (d, 2H, phenyl, $J = 8.7$ Hz), 7.38 (d, 2H, phenyl, $J = 8.7$ Hz), 7.63 (d, 2H, phenyl, $J = 8.7$ Hz), 7.76 (d, 2H, phenyl, $J = 8.4$ Hz), 8.34 (dd, 1H, pyridinium, $J = 7.2$ Hz), 9.17 (s, 1H, pyridinium), 9.20 (d, 1H, pyridinium, $J = 9.3$ Hz), 9.69 (s, 1H, pyridinium), 11.16 (s, 1H, CONH), 11.22 (s, 1H, CONH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 15.85$ (CH_3), 63.04 (CH_2), 121.47 (2 × CH), 121.79 (2 × CH), 127.41 (2 × CH), 127.73 (2 × CH), 128.26 (C), 128.27 (C), 129.6 (CH), 134.48 (C), 136.19 (C), 137.92 (C), 145.31 (CH), 147.42 (CH), 148.83 (CH), 160.81 (C=O), 163.89 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{21}H_{19}N_3O_2SCl$: 412.08810, found: 412.08811.

*1-Benzyl-3-carbamoyl-pyridinium chloride (**75**)* This compound was prepared from benzyl chloride (1.0 ml, 8.8 mmol) and nicotinamide (0.5 g, 4 mmol) to yield **75** as white solid (0.55 g, 54%); mp 217–218°C (decomp.), IR (KBr): $\nu_{max} = 3294$, 3147, 2939, 1697, 1581 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 5.97$ (s, 2H, CH_2), 7.42 (dd, 3H, $J = 4.2$, 4.8 Hz), 7.6 (dd, 2H, phenyl, $J = 4.5$, 7.8 Hz), 8.21 (s, 1H, $CONH_2$), 8.29 (dd, 1H, pyridinium, $J = 1.8$, 6.0 Hz), 8.67 (s, 1H, $CONH_2$), 8.99 (d, 1H, pyridinium, $J = 8.1$ Hz), 9.39 (d, 1H, pyridinium, $J = 6.0$ Hz), 9.72 (s, 1H, pyridinium) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 64.06$ (CH_2), 128.99 (CH), 129.70 (2 × CH), 129.77 (2 × CH), 129.90 (CH), 130.15 (CH), 134.69 (CH), 144.58 (CH), 145.47 (C, phenyl), 147.09 (C), 163.40 (C=O); HRMS-FAB *m/z* $[M]^+$ calcd for $C_{13}H_{13}N_2O$: 213.10279, found: 213.10224.

*3-Carbamoyl-1-phenethyl-pyridinium chloride (**76**)* This compound was prepared from phenyl ethyl chloride (1.0 ml, 7.6 mmol) and nicotinamide (0.5 g, 4 mmol) to yield **76** as yellowish pale solid (0.63 g, 60%); mp 204–206°C (decomp.), IR (KBr): $\nu_{max} = 3256$, 3125, 1689, 1643, 1581 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 3.30$ (t, 2H, CH_2, $J = 7.5$ Hz), 4.92 (t, 2H, $J = 7.2$ Hz), 7.27 (m, 5H, phenyl), 8.17 (s, 1H, $CONH_2$),, 8.23 (dd, 1H, pyridinium, $J = 1.8$, 6.6 Hz), 8.62 (s, 1H, $CONH_2$), 8.95 (d, 1H, pyridinium, $J = 8.1$ Hz), 9.19 (d, 1H, pyridinium, $J = 5.7$ Hz), 9.57 (s, 1H, pyridinium) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 37.03$ (CH_2), 62.49 (CH_2), 127.79 (CH), 128.35 (2 × CH), 129.32 (2 × CH), 129.64 (CH), 134.35 (C), 136.76 (C), 144.23 (CH), 145.52 (CH), 147.06 (CH), 163.43 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{14}H_{15}N_2O$: 227.11789, found: 227.11786.

*3-Carbamoyl-1-ethyloxycarbonylmethyl-pyridinium chloride (**77**)* This compound was prepared from 1-ethyl-oxycarbonylmethyl chloride (1.0 g, 8 mmol) and nicotinamide (0.5 g, 4 mmol) to yield **77** as yellowish

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irritant (lacrimant) solid (0.72 g, 79%); mp 151–153°C (decomp.), IR (KBr): $\nu_{max} = 3155, 3039, 1751, 1689, 1620\ cm^{-1}$, ^1H NMR (300 MHz, DMSO-d_6): $\delta = 1.23$ (t, 3H, CH_3, $J = 6.9$ Hz), 4.20 (q, 2H, CH_2, $J = 6.9$ Hz), 5.77 (s, 2H, CH_2), 8.21 (s, 1H, $CONH_2$),, 8.37 (dd, 1H, pyridinium, $J = 6.9$, 0 Hz), 8.66 (s, 1H, $CONH_2$), 9.58 (s, 1H, pyridinium), 9.24 (d, 1H, pyridinium, $J = 5.7$ Hz), 9.12 (d, 1H, pyridinium, $J = 8.1$ Hz) ppm; $_{13}C$ NMR (75 MHz, DMSO-d_6): $\delta =$ 14.62 (CH_3), 61.23 (CH_2), 63.09 (CH_2), 134.17 (C), 145.09 (CH), 147.36 (CH), 148.47 (CH), 163.37 (C=O), 166.91 (C=O) ppm; HRMS-FAB *m/z* $[M]^+$ calcd for $C_{10}H_{13}N_2O_3$: 209.09262, found: 209.09215.

Docking and scoring

The binding site was generated from the cocrystallized ligand BXZ1 within the targeted protein (PDB code: 3BM9, resolution 1.6 Å). The ligands were docked using LigandFit and default parameters (Venkatachalam *et al.*, 2003; Vieth *et al.*, 1998). The resulting docked poses were scored employing consensus scoring based on PLP1 (Gehlhaar *et al.*, 1995), PLP2 (Gehlhaar *et al.*, 1999), ligscore1 (Venkatachalam *et al.*, 2003), ligscore2 (Venkatachalam *et al.*, 2003), PMF (Böhm, 1994, 1998), and JAIN (Muegge and Martin, 1999; Muegge, 2000; Muegge, 2001). The optimal docked pose of BXZ1 achieved full consensus score from all six scoring functions, and therefore, the docked poses of thiamine and other synthetic pyridinium derivatives were scored employing consensus scoring.

In vitro experimental studies

Materials

All of the chemicals used in these experiments were of reagent grade and obtained from commercial suppliers. Thiamine (Sigma-Aldrich, purity >98%), Recombinant Human Hsp90α (BIOQUOTE, UK), ATP 100× solution (BIOQUOTE, UK), geldanamycin (BIOQUOTE, UK), Quantichrome ATPase/GTPase Kit (BioAssay Systems, USA), water for bioanalysis (Sigma, USA), DMSO for bioanalysis (Sigma, USA).

Preparation of hit compounds for In vitro assay

The synthesized compounds were kept as dry powders in variable quantities (50–100 mg). They were initially dissolved in DMSO to give stock solutions of 0.2 M. Subsequently, they were diluted to the required concentrations with deionized water for enzymatic assay.

Quantification of Hsp90α activity in a spectrophotometric assay

The kinase activity of Hsp90α was quantified by colorimetric measurement of released inorganic phosphate. Bioassays were performed by mixing Hsp90α solution (6 µl, 25 µg/ml in assay buffer), 24 µl assay buffer, and 5 µl of the particular tested compounds to yield final inhibitor concentrations of 100, 10, and 1 µM per well. The final concentration of DMSO did not exceed 1.0%. The mixtures were incubated for 30 min at 37°C in ELISA plate shaker, and then ATP solutions (5 µl, 4 mM in assay buffer) were added to each mixture. 5 µl of 80% CMC (Critical Micelle Concentration) of chaps was used for authenticity and elimination of promiscuous effect. The volume was completed to 40 µl using kinase assay buffer. Blank was prepared as above except 5 µl of distilled water was used instead of inhibitor solution. The mixtures were equilibrated to 37°C and incubated for 24 h. The enzymatic reaction was terminated by the addition of 80 µl malachite green ammoniummolybdate–tween 20 solutions in 0.27 M H_2SO_4 and 10 µl of 34% Na citrate. Color was allowed to develop at room temperature for 30 min, and sample absorbance were determined at λ_{max} 620 nm using a plate reader (Bio-Tek instruments ELx 800, USA).

Inhibition of recombinant Hsp90α was calculated as percentage activity of the uninhibited kinase control. Geldanamycin was tested as positive control, while negative controls were prepared by adding the substrate after reaction termination (Lanzetta *et al.*, 1979; Avila *et al.*, 2006a, b).

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References

Avila C, Boris AK, Brian SJ (2006a) Development and optimization of a useful assay for determining Hsp90s inherent ATPase activity. Bioorg Med Chem 14:1134–1142

Avila C, Kyle HM, Zeqiang M, Boris AK, Qi-Zhuang Y, Brian SB (2006b) High-throughput screening for Hsp90 ATPase inhibitors. Bioorg Med Chem Lett 16:3005–3008

Böhm HJ (1994) The development of a simple empirical scoring function to estimate the binding constant for a protein-ligand complex of known three-dimensional structure. J Comput-Aided Mol 8:243–256

Böhm HJ (1998) Prediction of binding constants of protein ligands: a fast method for the prioritization of hits obtained from de novo design or 3D database search programs. J Comput-Aided Mol 12:309–323

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MS Code : MCRE-915 | ☑ CP | ☑ DISK

Chiosis G, Lucas B, Shtil A, Huezo H, Rosen N (2002) Development of a purine-scaffold novel class of Hsp90 binders that inhibit the proliferation of cancer cells and induce the degradation of Her2 tyrosine kinase. Bioorg Med Chem 10:3555–3564

Chiosis G, Rodina A, Moulick K (2006) Emerging Hsp90 inhibitors: from discovery to clinic. Anticancer Agents Med Chem 6:1–8

Christopher R, Edwards W, Bouchier IA (Eds) (1991) Oncology, chapter seven, Davidson's principles and practice of medicine, 16th edn. ELBS Publisher, UK

ComõÂn-Anduix B, Boren J, Martinez S, Moro C, Centelles JJ, Trebukhina R, Petushok N, Lee W-N P, Boros LG, Cascante M (2001) The effect of thiamine supplementation on tumour proliferation: a metabolic control analysis study. Eur J Biochem 268:4177

Dey A, Cederbaum AI (2007) Geldanamycin, an inhibitor of Hsp90 increases cytochrome P450 2E1 mediated toxicity in HepG2 cells through sustained activation of the p38MAPK pathway. Arch Biochem Biophys 461:275–286

Gehlhaar DK, Verkhivker GM, Rejto PA, Sherman CJ, Fogel DB, Fogel LJ, Freer ST (1995) Molecular recognition of the inhibitor AG-1343 by HIV-1 protease: conformationally flexible docking by evolutionary programming. Chem Biol 2:317–332

Gehlhaar DK, Bouzida D, Rejto PA (1999) In: Parrill L, Reddy MR, (eds) Rational drug design: novel methodology and practical applications. American Chemical Society, Washington, DC, pp 292–311

Gopalsamy A, Shi M, Golas J, Vogan E, Jacob J, Johnson M, Lee F, Nilakantan R, Petersen R, Svenson K, Chopra R, Tam MS, Wen Y, Ellingboe J, Arndt K, Boschelli F (2008) Discovery of benzisoxazoles as potent inhibitors of chaperone heat shock protein 90. J Med Chem 51:373–375

Hideyuki O, Masami K, Yuichi T, Yumiko U, Jun F, Takayuki N, Shiro S, Makoto S, Shunichi I, Yoshinori Y, Endang SR, Yutaka K, Michio I (2008) Conformational significance of EH21A1–A4, phenolic derivatives of geldanamycin, for Hsp90 inhibitory activity. Bioorg Med Chem Lett 18:1577–1580

Kasibhatla SR, Hong K, Biamonte MA, Busch DJ, Karjian PL, Sensintaffar JL, Kamal A, Lough RE, Brekken J, Lundgren K, Grecko R, Timony GA, Ran Y, Mansfield R, Fritz LC, Ulm E, Burrows FJ, Boehm MF (2007) Rationally designed high-affinity 2-amino-6-halopurine heat shock protein 90 inhibitors that exhibit potent antitumor activity. J Med Chem 50:2767–2778

Lanzetta PA, Alvarez LJ, Reinach PS, Candia OA (1979) An improved assay for nanomole amounts of inorganic phosphate. Anal Biochem 100:95–97

Muegge I (2000) A knowledge-based scoring function for protein-ligand interactions: probing the reference state. Perspect Drug Discov Des 20:99–114

Muegge I (2001) Effect of ligand volume correction on PMF scoring. J Comput Chem 22:418–425

Muegge I, Martin YC (1999) A general and fast scoring function for protein-ligand interactions: a simplified potential approach. J Med Chem 42:791–804

Neckers L, Mollapour M, Tsutsumi S (2009) The complex dance of the molecular chaperone Hsp90. Trends Biochem Sci 34: 223–226

Prodromou C, Pearl LH (2003) Structure and functional relationships of Hsp90. Curr Cancer Drug Targets 3:301–323

Rowlands MG, Newbatt YM, Prodromou C, Pearl LH, Workman P, Aherne W (2004) High-throughput screening assay for inhibitors of heat-shock protein 90 ATPase activity. Anal Biochem 327:176–183

Solit DB, Rosen N (2006) Hsp90: a novel target for cancer therapy. Curr Top Med Chem 6:1205–1214

Venkatachalam CM, Jiang X, Oldfield T, Waldman M (2003) Ligandfit: a novel method for the shape-directed rapid docking of ligands to protein active sites. J Mol Graph Model 21:289–307

Vieth M, Hirst JD, Dominy BN, Daigler H, Brooks CL III (1998) Assessing search strategies for flexible docking. J Comput Chem 19:1623–1631

Xiao L, Lu X, Ruden DM (2006) Effectiveness of Hsp90 inhibitors as anti-cancer drugs. Mini-Rev Med Chem 6:1137–1143

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