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Short communication

Synthesis, SAR and antibacterial studies on novel chalcone oxazolidinone hybrids☆

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Abstract

With an intention to synergise the antibacterial activity of chalcones and oxazolidinones, several hybrid compounds possessing both chalcone and oxazolidinone moieties were synthesized and tested for antibacterial activity. The hybrid molecules containing heterocycles instead of aromatic ring were found to be active. A SAR study with various heterocycles resulted in a lead molecule **20**, which was converted to one of the potent antibacterial compounds **27**.

Keywords: Oxazolidinones; Antibacterial; Chalcones; Linezolid

1. Introduction

The emergence of multi-drug-resistant Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermitis* (MRSE) and vancomycin resistant enterococci (VRE) is of major concern [1–5]. Oxazolidinones, exemplified by DuP-721 [6], are a new class of synthetic antibacterials with activity against Gram-positive bacteria and anaerobic bacteria, including resistant pathogens. As chalcone compounds are known to be effective antibacterial compounds [7], we initiated a program to synergise the antibacterial activity of both chalcones and oxazolidinones by preparing hybrid molecules having the features of both oxazolidinones and chalcones in an effort to discover potent antibacterials. In this paper, we describe a systematic SAR study performed in this area that resulted in identification of a lead molecule that was converted to one of the potent molecules following the protocols developed during our earlier efforts [8].

DuP-721 **1** was a potent oxazolidinone antibacterial developed by DuPont [9]. In a hypothetical hybridization of **1** with the chalcone structure **2**, there resulted two chalcone–oxazolidinone hybrid structures **3** and **4** (Diagram 1). The hybrid structure **3** possessing the carbonyl group away from oxazolidinone ring is called Type A hybrid molecules and the structure **4** possessing the carbonyl group close to oxazolidinone ring is called Type B hybrids. Both regioisomeric chalcone–oxazolidinone hybrid molecules were synthesized and studied for antibacterial activity.

2. Chemistry

The aldehyde intermediate **5**, served as the starting material for Type A hybrid molecules, was prepared as per our reported protocol [10]. The starting materials, DuP-721 **1** [9] and its fluorinated compound **17** [11], for Type B hybrid molecules were prepared as per literature reports. All the chalcone molecules described in Table 1 were synthesized from the

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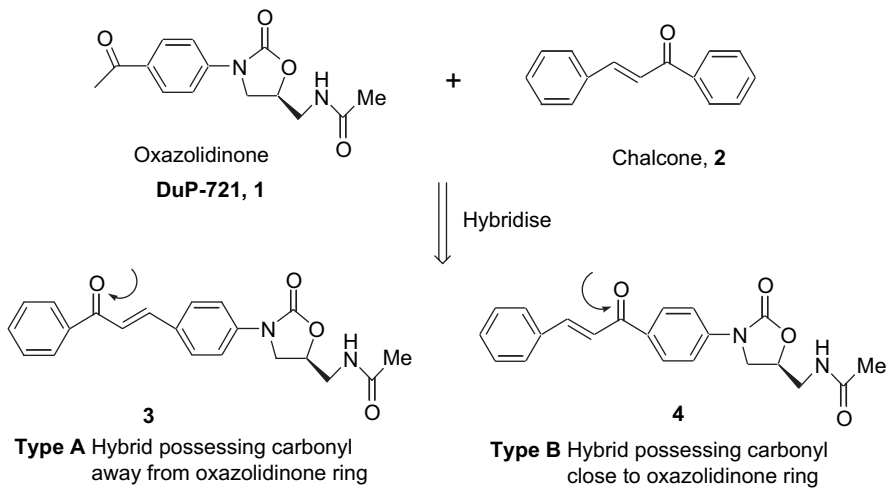


Diagram 1.

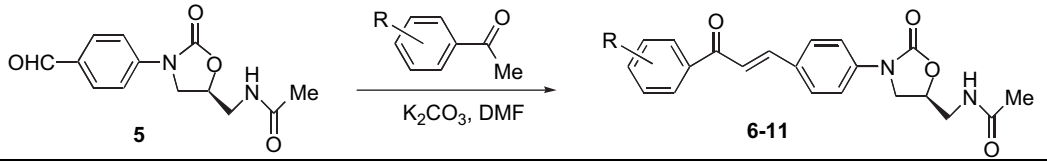
oxazolidinone aldehyde **5** by aldol condensation with respective acetophenones under basic conditions. The compounds **8**, **9** and **10** were obtained as inseparable mixtures of geometrical isomers. Analogously, the compounds appearing in Table 2 were obtained by aldol condensation of DuP-721 **1** with respective aromatic aldehydes using identical reaction conditions. Appropriate heterocyclic aldehydes were employed in aldol condensation of either DuP-721 **1** or the compound **17** in the preparation of compounds illustrated in Table 3.

The thiocarbamate compound **27** was synthesized from the intermediate **17** as depicted in Scheme 1 following a modified condition for the requisite aldol condensation. Initially, the acetamide intermediate **17** was converted to the corresponding thiocarbamate **27** by hydrolysis to the amine **25** followed by conversion of the amine functionality to the thiocarbamate **26** under standard conditions. The attempted aldol condensation of the thiocarbamate **26** with 2-pyridine carboxaldehyde under usual conditions did not result in the requisite chalcone **27** presumably due to the interference of the thiocarbamte functionality. Consequently, the aldol reaction was attempted with a variety of bases of which LDA was found to be suitable affording the required thiocarbamate **27** in modest yield.

3. Results and discussion

The chalcone–oxazolidinone hybrid molecules prepared above were screened for *in vitro* activity against a panel of Gram-positive organisms and the results are summarized in Tables 1 and 2. The Type A hybrid molecules **6–11** were designed in such a way that the aromatic ring would have a substituent starting from strong electron donating to strong electron withdrawing nature (Table 1). They exhibited only trace or no activity against all the organisms tested. Similarly, the Type B hybrid molecules **12–16** (Table 2) possessing various substituents on the aromatic ring were also inactive. However, moderate activity was observed for compounds **18** and **19** when the aromatic ring of the chalcone was replaced with heterocycles (entries 1–2,

Table 1
In vitro antibacterial activity (MIC, μg/mL)[a] of Type A hybrid molecules[b]

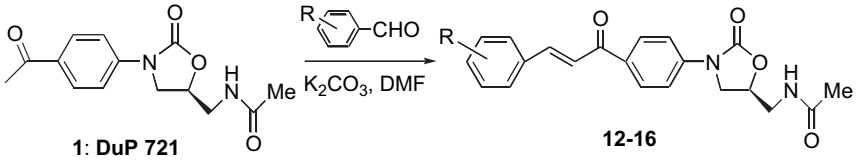


S. no.	Compound	R	S.a 019	S.a 213	S.a 035	E.f 034	E.f 153	E.fm 154
1	**6**	4-OMe	>32	>32	>32	>32	>32	>32
2	**7**	4-Me	32	32	>32	32	32	32
3	**8**	H	>32	>32	>32	>32	>32	>32
4	**9**	3-F	64	>64	>64	>64	>64	>64
5	**10**	4-NO_2	32	32	32	32	32	32
6	**11**	2,4-$(Cl)_2$-6-F	>32	>32	>32	>32	>32	>32

[a] S.a 019 = *Staphylococcus aureus* ATCC 33591 (methicillin-resistant); S.a 213 = *Staphylococcus aureus* ATCC 49951; S.a 035 = *Staphylococcus aureus* ATCC 29213; E.f 034 = *Enterococcus faecalis* ATCC 29212 (vancomycin sensitive); E.f 153 = *Enterococcus faecalis* NCTC 12201 (vancomycin resistant) and E.fm 154 = *Enterococcus faecium* ATCC 12202 (vancomycin resistant).

[b] Compounds exist as a mixture of *E* and *Z* isomers.

Table 2
In vitro antibacterial activity (MIC, μg/mL)[a] of Type B hybrid molecules



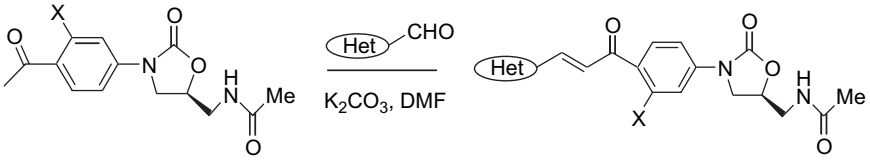
S. no.	Compound	R or heterocycle	S.a 019	S.a 213	S.a 035	E.f 034	E.f 153	E.fm 154
1	**12**	3,4,5-$(OMe)_3$	>32	>32	>32	>32	>32	>32
2	**13**	3,4-$(OMe)_2$	>32	>32	>32	>32	>32	>32
3	**14**	H	>32	>32	>32	>32	>32	>32
4	**15**	4-F	>32	>32	>32	>32	>32	>32
5	**16**	4-COOMe	>32	>32	>32	>32	>32	>32

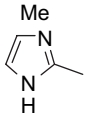
[a] See footnote 'a' of Table 1 for details about the organisms.

Table 3 at this stage, it was envisioned that introducing a fluorine atom in the aromatic ring would enhance the activity. Indeed, these heterocycle substituted hybrid molecules **20** and **21** showed better antibacterial activity than the non-fluorine analogues (entries 3–4, Table 3). But, the introduction of thiophene and imidazole heterocycles resulted in compounds **22**–**24** that were inferior in activity. In this SAR study, compound **20** possessing a pyridine ring was found to be the best molecule showing acceptable antibacterial activity (MIC 4 μg/mL) against an MRSA strain.

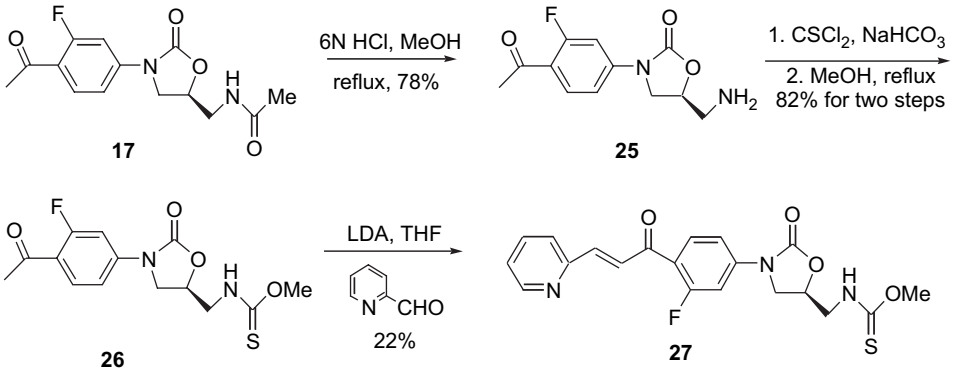
Having found a molecule exhibiting moderate activity, we used our experience of converting the acetamide group of **20**

Table 3
In vitro antibacterial activity (MIC, μg/mL)[a] of Type B hybrid molecules possessing heterocycles



S. no.	Compound	R or heterocycle	S.a 019	S.a 213	S.a 035	E.f 034	E.f 153	E.fm 154
1	**18**		32	32	32	64	64	64
2	**19**		32	16	16	32	32	32
3	**20**		4	4	8	16	16	16
4	**21**		8	8	8	16	16	16
5	**22**		32	32	32	32	32	32
6	**23**		32	32	32	32	32	32
7	**24**		16	16	16	16	16	16
	Linezolid		1	2	2	2	2	2
	Vancomycin		2	1	1	2	>32	>32

[a] See footnote 'a' of Table 1 for details about the organisms.



Scheme 1. Synthesis of thiocarbamate compound **27**.

to the corresponding thiocarbamate [8]. The resultant molecule **27** exhibited *in vitro* activity in the range of 0.25–2 μg/mL (Table 4). It is important to note that this compound **27** is many fold superior in activity when compared to its acetamide analogue **20**.

4. Conclusion

A systematic SAR study in the novel area of chalcone–oxazolidinone hybrids was undertaken. Although both the Type A and Type B hybrid molecules were inactive, the introduction of heterocycles instead of an aromatic ring led to new molecules possessing moderate *in vitro* activity. Introduction of a variety of heterocycles resulted in a lead molecule **20** possessing a pyridine-aromatic hybrid structure. Based on our experience in the area of oxazolidinones, this moderately active molecule **20** was transformed to one of the potent compounds **27**.

5. Experimental protocols

Melting points are uncorrected. IR spectra were recorded on a Perkin–Elmer 1650 spectrophotometer. All ^{1}H NMR were recorded at 200 MHz on a Varian Gemini spectrometer. Chemical shifts are reported in δ units with respect to TMS as internal standard. Mass spectra were recorded on a HP-5989A spectrometer. All HPLC′s were run with a system that consisted of Hichrom RPB column (250 mm), 0.01 M KH_2PO_4/CH_3CN 50:50 at 1 mL/min flow rate, 220 nm.

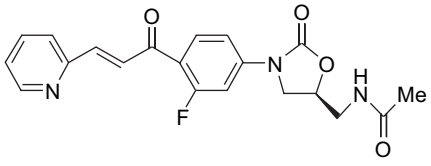
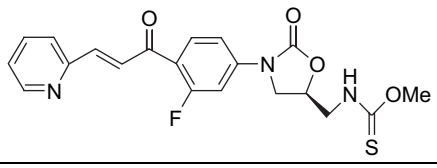
5.1. General procedure for the synthesis of chalcones

A suspension of an appropriate acetophenone (1 mmol), the corresponding benzaldehyde (1 mmol) and K_2CO_3 (3 mmol) in dry DMF (5 mL) was stirred at rt. The reaction was monitored by TLC and was warmed to 60–80 °C if required. The reaction mixture was worked up, upon completion of the reaction as judged by TLC, by adding water and extracting with ethyl acetate. The combined organic extracts were washed with water, brine and dried. The residue obtained upon evaporation of solvent was chromatographed over silica gel to afford the required product. The following chalcone molecules were prepared using this general procedure.

5.1.1. *N*-(3-{4-[3-(4-Methoxy-phenyl)-3-oxo-propenyl]-phenyl}-2-oxo-oxazolidin-5-ylmethyl)-acetamide **6**

Yield: 72%; m.p. 174 °C; IR (KBr): 1750, 1658, 1523 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.04 (d, $J = 8.8$ Hz, 1H),

Table 4
Comparison of *in vitro* antibacterial activity (MIC, μg/mL)[a] of **20** with **27**

S. no.	Compound	Structure	S.a 019	S.a 213	S.a 035	E.f 034	E.f 153	E.fm 154
1	**20**		4	4	8	16	16	16
2	**27**		0.25	1	0.5	2	1	2

[a] See footnote 'a' of Table 1 for details about the organisms.

7.88–7.26 (m, 8H), 6.98 (d, $J = 8.8$ Hz, 1H), 6.10 (bs, 1H), 4.79 (m, 1H), 4.10 (t, $J = 8.8$ Hz, 1H), 3.89 and 3.86 (2s, 3H), 3.85–3.67 (m, 3H), 2.02 (s, 3H); MS (DIP): 394, 350, 266; HPLC: 88.2% purity.

5.1.2. N-{2-Oxo-3-[4-(3-oxo-3-p-tolyl-propenyl)-phenyl]-oxazolidin-5-ylmethyl}-acetamide ***7***

Yield: 53%; m.p. 195 °C; IR (KBr): 1745, 1655, 1517, 1228 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.94 (d, $J = 7.8$ Hz, 1H), 7.58–7.22 (m, 9H), 6.07 (bs, 1H), 4.80 (bs, 1H), 4.10 (t, $J = 8.8$ Hz, 1H), 3.83 (t, $J = 8.8$ Hz, 1H), 3.72–3.56 (m, 2H), 2.44 and 2.39 (2s, 3H), 2.03 (s, 3H); MS (DIP): 378 (M^+), 334, 250, 119; HPLC: 89.6% purity.

5.1.3. N-{2-Oxo-3-[4-(3-oxo-3-phenyl-propenyl)-phenyl]-oxazolidin-5-ylmethyl}-acetamide ***8***

Yield: 59%; m.p. 182 °C; IR (KBr): 1750, 1678, 1517 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.04–7.40 (m, 10H), 6.03 (bs, 1H), 4.82 (bs, 1H), 4.18–3.20 (m, 4H), 2.02 (s, 3H); MS (CI-method): 365 ($M^+ + 1$), 277, 263; HPLC: 93.0% purity.

5.1.4. N-(3-{4-[3-(3-Floro-phenyl)-3-oxo-propenyl]-phenyl}-2-oxo-oxazolidin-5-ylmethyl)-acetamide ***9***

Yield: 75%; m.p. 165 °C; IR (neat): 1751, 1684, 1588, 1435 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.74–7.25 (m, 10H), 6.02 (bs, 1H), 4.80 (bs, 1H), 4.02 (t, $J = 8.8$ Hz, 1H), 3.77–3.27 (m, 3H), 2.00 (s, 3H); MS (CI-method): 383 ($M^+ + 1$), 339, 139; HPLC: 86.9% purity.

5.1.5. N-(3-{4-[3-(4-Nitro-phenyl)-3-oxo-propenyl]-phenyl}-2-oxo-oxazolidin-5-ylmethyl)-acetamide ***10***

Yield: 37%; m.p. 190 °C; IR (neat): 1754, 1674, 1580 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 8.38–8.07 (m, 4H), 7.85–7.26 (m, 6H), 4.80 (bs, 1H), 4.11–3.43 (m, 4H), 1.99 (s, 3H); MS (CI-method): 410 ($M^+ + 1$), 336, 166; HPLC: 90.1% purity.

5.1.6. N-(2-Oxo-3-{4-[3-(3,4,5-trimethoxy-phenyl)-acryloyl]-phenyl}-oxazolidin-5-ylmethyl)-acetamide ***12***

Yield: 76%; m.p. 225 °C; IR (KBr): 1760, 1649, 1459 cm^{-1}; ^1H NMR (DMSO-d_6): δ 8.25–7.72 (m, 7H), 7.24 (s, 2H), 4.80 (bs, 1H), 4.21 (m, 1H), 3.88–3.45 (m, 12H), 1.83 (s, 3H); MS (CI-method): 455 ($M^+ + 1$), 411; HPLC: 92.7% purity.

5.1.7. N-(3-{4-[3-(3,4-Dimethoxy-phenyl)-acryloyl]-phenyl}-2-oxo-oxazolidin-5-ylmethyl)-acetamide ***13***

Yield: 68%; m.p. 216 °C; IR (KBr): 1765, 1650, 1456 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.07 (d, $J = 8.8$ Hz, 2H), 7.80–7.10 (m, 6H), 6.91 (d, $J = 8.3$ Hz, 1H), 6.04 (bs, 1H), 4.90 (bs, 1H), 4.11 (t, $J = 8.8$ Hz, 1H), 3.96 (s, 3H), 3.94 (s, 3H), 3.93–3.68 (m, 3H), 2.03 (s, 3H); MS (CI-method): 425 ($M^+ + 1$), 381, 277, 235; HPLC: 99.9% purity.

5.1.8. N-{2-Oxo-3-[4-(3-phenyl-acryloyl)-phenyl]-oxazolidin-5-ylmethyl}-acetamide ***14***

Yield: 49%; m.p. 230 °C; IR (KBr): 1735, 1653, 1409, 1230 cm^{-1}; ^1H NMR (DMSO-d_6): δ 8.25–7.40 (m, 12H), 4.80 (bs, 1H), 4.30–3.20 (m, 4H), 1.84 (s, 3H); MS (CI-method): 365 ($M^+ + 1$), 321, 235; HPLC: 85.6% purity.

5.1.9. N-(3-{4-[3-(4-Fluoro-phenyl)-acryloyl]-phenyl}-2-oxo-oxazolidin-5-ylmethyl)-acetamide ***15***

Yield: 81%; m.p. 252 °C; IR (KBr): 1734, 1654, 1586, 1224 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 8.07 (d, $J = 8.8$ Hz, 1H), 7.90–7.20 (m, 8H), 7.13 (t, $J = 8.8$ Hz, 1H), 4.95–4.70 (m, 1H), 4.18–4.83 (m, 2H), 3.61–3.59 (m, 2H), 1.98 (s, 3H); MS (CI-method): 383 ($M^+ + 1$), 277, 235; HPLC: 99.7% purity.

5.1.10. N-(3-{4-[3-(4-Carbomethoxy-phenyl)-acryloyl]-phenyl}-2-oxo-oxazolidin-5-ylmethyl)-acetamide ***16***

Yield: 40%; m.p. 250 °C; IR (KBr): 1733, 1654, 1607, 1283 cm^{-1}; ^1H NMR (DMSO-d_6): δ 8.40–7.70 (m, 10H), 4.90–4.70 (m, 1H), 4.23 (t, $J = 8.8$ Hz, 1H), 3.89 (s, 3H), 4.00–3.20 (m, 3H), 1.84 (s, 3H); MS (CI-method): 423 ($M^+ + 1$), 379; HPLC: 96.5% purity.

5.1.11. N-{2-Oxo-3-[4-(3-pyridin-2-yl-acryloyl)-phenyl]-oxazolidin-5-ylmethyl}-acetamide ***18***

Yield: 73%; m.p. 180 °C; IR (KBr): 1740, 1657, 1596, 1221 cm^{-1}; ^1H NMR (DMSO-d_6): δ 8.70 (d, $J = 4.4$ Hz, 1H), 8.27–8.14 (m, 4H), 7.91 (d, $J = 4.0$ Hz, 2H), 7.77–7.69 (m, 3H), 7.50 (dd, $J = 4.4$ and 8.8 Hz, 1H), 4.81–4.70 (m, 1H), 4.21 (t, $J = 9.3$ Hz, 1H), 3.84 (t, $J = 7.3$ Hz, 1H), 3.45 (t, $J = 4.8$ Hz, 2H), 1.84 (s, 3H); MS (CI-method): 366 ($M^+ + 1$), 321, 237, 132; HPLC: 95.4% purity.

5.1.12. N-{3-[4-(3-Furan-2-yl-acryloyl)-phenyl]-2-oxo-oxazolidin-5-ylmethyl}-acetamide ***19***

Yield: 71%; m.p. 195 °C; IR (KBr): 1769, 1649, 1604 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 8.20–7.20 (m, 6H), 6.55 (bs, 3H), 6.12 (bs, 1H), 4.90–4.80 (m, 1H), 4.20–3.50 (m, 4H), 2.03 (s, 3H); MS (CI-method): 354 ($M^+ + 1$), 310, 226; HPLC: 97.2% purity.

5.1.13. N-{3-[3-Fluoro-4-(3-pyridin-2-yl-acryloyl)-phenyl]-2-oxo-oxazolidin-5-ylmethyl}-acetamide ***20***

Yield: 61%; m.p. 170 °C; IR (KBr): 1744, 1620, 1218 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.69 (d, $J = 4.4$ Hz, 1H), 8.00–7.20 (m, 8H), 6.10 (bs, 1H), 4.90–4.70 (m, 1H), 4.11 (t, $J = 8.8$ Hz, 1H), 3.85 (t, $J = 7.3$ Hz, 1H), 3.72–3.65 (m, 2H), 2.04 (s, 3H): MS (CI-method): 385 ($M^+ + 1$), 340; HPLC: 98.5% purity.

5.1.14. N-{3-[3-Fluoro-4-(3-furan-2-yl-acryloyl)-phenyl]-2-oxo-oxazolidin-5-ylmethyl}-acetamide ***21***

Yield: 83%; m.p. 120 °C; IR (KBr): 1744, 1618, 1404, 1222 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.92 (t, $J = 8.3$ Hz, 1H), 7.63–7.20 (m, 4H), 7.38 (d, $J = 2.9$ Hz, 1H), 6.74 (d, $J = 2.9$ Hz, 1H), 6.52 (bs, 1H), 6.01 (bs, 1H), 4.90–4.75 (m,

1H), 4.11 (t, $J = 9.3$ Hz, 1H), 3.84 (t, $J = 6.8$ Hz, 1H), 3.71–3.65 (m, 2H), 2.04 (s, 3H); MS (CI-method): 373 ($M^+ + 1$), 253; HPLC: 97.4% purity.

5.1.15. N-{3-[3-Fluoro-4-(3-thiophen-2-yl-acryloyl)-phenyl]-2-oxo-oxazolidin-5-ylmethyl)-acetamide ***22***

Yield: 66%; m.p. 130 °C; IR (KBr) 1745, 1656, 1618, 1402 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.00–7.80 (m, 2H), 7.64–7.21 (m, 5H), 7.09 (t, $J = 4.4$ Hz, 1H), 6.03 (bs, 1H), 4.90–4.70 (m, 1H), 4.11 (t, $J = 8.8$ Hz, 1H), 3.84 (t, $J = 6.8$ Hz, 1H), 3.72–3.49 (m, 2H), 2.04 (s, 3H); MS (CI-method): 389 ($M^+ + 1$), 345, 295; HPLC: 98.7% purity.

5.1.16. N-(3-{3-Fluoro-4-[3-(1-methyl-1H-imidazol-2-yl)-acryloyl]-phenyl}-2-oxo-oxazolidin-5-ylmethyl)-acetamide ***23***

Yield: 48%; m.p. 208 °C; IR (neat) 3345, 1752, 1614, 1408 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 8.10–8.00 (m, 2H), 7.67–7.62 (m, 3H), 7.37–7.32 (m, 2H), 4.84 (bs, 1H), 4.13 (t, $J = 8.8$ Hz, 1H), 3.91 (m, 1H), 3.92 (s, 3H), 3.60–3.57 (m, 2H), 1.97 (s, 3H); MS (CI-method): 387 ($M^+ + 1$), 343, 111; HPLC: 99.6% purity.

5.1.17. N-{3-[3-Fluoro-4-(3-1H-imidazol-2-yl-acryloyl)-phenyl]-2-oxo-oxazolidin-5-ylmethyl}-acetamide ***24***

Yield: 58%; m.p. 224 °C; IR (KBr) 1755, 1622, 1405 cm^{-1}; ^1H NMR (DMSO-d_6): δ 8.28 (bs, 1H), 7.88 (t, $J = 8.3$ Hz, 1H), 7.68–7.20 (m, 6H), 4.90–4.70 (m, 1H), 4.19 (t, $J = 6.8$ Hz 1H), 3.81 (t, $J = 6.8$ Hz, 1H), 3.60–3.00 (m, 4H), 1.83 (s, 3H); MS (CI-method): 373 ($M^+ + 1$), 353, 309; HPLC: 96.1% purity.

5.1.18. {3-[3-Fluoro-4-(3-pyridin-2-yl-acryloyl)-phenyl]-2-oxo-oxazolidin-5-ylmethyl}-thiocarbamicacid O-methylester ***27***

A solution of the acetamide **17** (300 mg, 1.1 mmol) in 6 N HCl (5 mL) was refluxed over a period of 2 h. The reaction mixture was allowed to cool to rt, neutralized with aq. $NaHCO_3$ and extracted with ethyl acetate. The combined organic extracts were washed with water, brine and dried. The residue obtained upon evaporation of solvent to afford the amine **25**, which was directly used in the next step. Thiophosgene (150 mg, 1.3 mmol) was added dropwise to a solution of the above amine **25** and Et_3N (0.37 mL, 2.6 mmol) in dry dichloromethane at ice bath temperature under argon. The reaction mixture was warmed to rt over 3 h and then the volatiles were removed to obtain the corresponding isothiocyanate. The crude isothiocyanate in methanol was heated to 80–100 °C while monitoring by TLC. At the complete consumption of starting material, the reaction mixture was allowed to cool to rt. The crystals formed were separated, washed with ether and dried under vacuum to yield the thiocarbamate **26**.

To a solution of LDA (0.14 mmol), prepared by the addition of diisopropylamine (20 μL, 0.14 mmol) to a solution of *n*-BuLi (1.6 M in hexanes, 88 μL, 0.14 mmol) in dry THF (2 mL) at −78 °C under argon, was added a solution of the thiocarbamate **26** (40 mg, 0.13 mmol) in dry THF (0.5 mL). After stirring the reaction mixture for 30 min at the same temperature, a solution of pyridine-2-carboxaldehyde (15 mg, 0.14 mmol) in dry THF (0.5 mL) was added. The reaction mixture was allowed to warm to rt and stirred for further 12 h before being quenched with saturated NH_4Cl solution. The resultant mixture was extracted with ethyl acetate. The combined organic extracts were washed with water, brine and dried. The residue obtained upon evaporation of solvent was chromatographed on silica gel to obtain the thiocarbamate **27** (12 mg, 22%) as an off-white solid. M.p. 139 °C; IR (KBr): 2923, 1753, 1620, 1512 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.69 (t, $J = 3.8$ Hz, 1H), 8.00–7.20 (m, 8H), 6.80–6.60 (bs, 1H), 5.05–4.90 (m, 1H), 4.20–3.90 (m, 4H), 4.01 (s, 3H); MS (CI-method) 372, 283, 110; HPLC: 89.1% purity.

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