Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 2501–2504

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

# Parallel Synthesis of Isatin-Based Serine Protease Inhibitors

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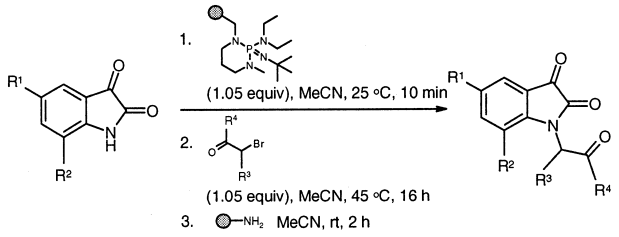
Received 19 July 2000; accepted 30 August 2000

**Abstract**—The synthesis of *N*-functionalised isatins using parallel, solution synthesis is described. Functionalised polymers were employed as stoichiometric and catalytic reagents as well as purification media in the exercise, and the derivatives were screened against a panel of serine proteases; high percentage inhibition was observed in several cases. 

As part of ongoing investigations into targeted libraries based on privileged structural motifs, we turned our attention to isatin. Over recent years, isatin derivatives have been studied as putative acyl traps for serine and cysteine proteases. In 1995, Iyer and co-workers reported that simple *N*-BOC and *N*-CBZ-isatin analogues acted as reversible, slow-binding inhibitors of serine proteases, and exhibited selectivity for α-chymotrypsin over porcine pancreatic elastase.[1] Webber and co-workers at Agouron subsequently outlined a comprehensive and successful study of isatin-derived inhibitors of HRV 3C protease.[2] In view of the evident potential of isatin to serve as a biologically-relevant motif for serine and cysteine proteases, we set out to make a series of arrays that could serve as probes for these families of enzyme targets. We chose to prepare a variety of analogues, ranging from simple *N*-functionalised isatins to more complex derivatives, and in all cases employed a solution chemistry approach that was amenable to the rapid, parallel synthesis and purification of at least 10 milligram quantities of material.

Scheme 1 outlines the method used for preparing of *N*-alkylated isatins **1**–**10**. For the initial deprotonation step, we judged that use of a polymer-supported strong base would be more suitable to high throughput parallel synthesis than sodium hydride; accordingly, we treated a series of 5- and 5,7-functionalised isatins with 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine[3] on polystyrene (BEMP, 2.3 mmol/g loading, 1.05 equiv) in acetonitrile, and quenched the resulting anion with α-bromoketone (1.05 equiv). The reaction mixtures were agitated at 45 °C for 16 h, after which the crude products were treated with a functionalised amine resin to remove any unreacted electrophile.[4]




**Scheme 1.** Preparation of *N*-alkylated isatin analogues.

Supported BEMP has been used successfully as a strong base by other researchers in similar contexts.[5,6] During our method development studies, we observed complete consumption of starting isatin using BEMP as the base, and isolated yields of desired *N*-alkylated products were high, typically exceeding 90% following polymeric scavenging of excess electrophile. When we examined the method using parallel synthesis, we obtained similar encouraging results: complete conversion of isatins was observed, and the desired products were isolated in similarly high yields, generally with good levels of chemical purity as determined by $^1$H NMR and HPLC-MS analysis.[7,8] Random examples of the members of this array are summarised in Table 1.

We then extended this exercise to the preparation of an additional series of isatin analogues based loosely on the neutrophil elastase inhibitors developed by Zeneca in the mid-1990s.[9] As for the approach outlined above, we chose to prepare the analogues in solution using

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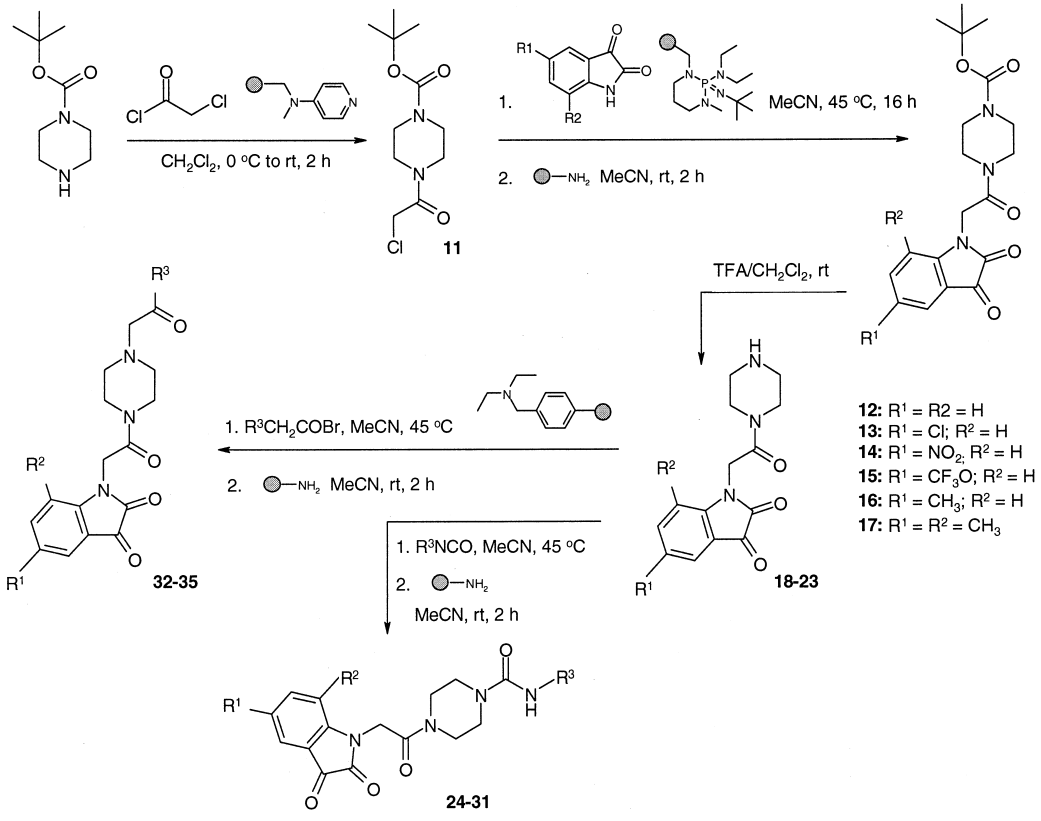
0960-894X/00/$ - see front matter 
PII: S0960-894X(00)00523-0

supported reagents. 1,4-Piperazine was selected as the core heterocycle in this study owing to its ubiquitous use in the area of protease inhibitors.[10] The piperazine moiety was attached to the isatin scaffold via a simple acetyl linker. Reactive electrophiles—excesses of which could be removed by polymeric scavenging—were chosen to allow for the incorporation of a diverse range of nitrogen capping groups, which encompassed polar and lipophilic motifs. Scheme 2 outlines the synthetic approach adopted, and results obtained in the exercise are listed in Table 2.

Initially, mono-BOC-protected piperazine (1.0 weight) in dichloromethane at 0 °C was treated with chloracetyl chloride (1.05 equiv) and polymer-supported DMAP (10 mol%, 2.0 mmol/g loading). The reaction mixture was warmed to room temperature over 2 h, after which the resin was removed by filtration. The resulting crude material was rapidly purified on a cartridge pre-packed with normal-phase silica[11] using a 20-channel vacuum manifold,[12] eluting with ethyl acetate:hexanes (3:1). This allowed for **11** to be isolated on a gram scale as a white solid in 90% yield.[13] Compound **11** was then treated with isatin anions, generated as outlined above using polymeric BEMP in acetonitrile, and, after 16 h at 45 °C, the solutions were cooled to room temperature and treated with functionalised amine resin to remove any unreacted starting material. The resulting isatin analogues, **12**–**17**, were isolated in purities exceeding 90%, again following rapid purification through pre-packed silica cartridges.[11] These analogues were prepared on a 100 mg scale, and were analysed by 400 MHz $^1$H NMR and LCMS.[14] Consumption of starting material was monitored in all cases, and isolated yields of all analogues exceeded 80%. Liberation of the corresponding free bases, **18**–**23**, was accomplished in quantitative yield using trifluoroacetic acid (50%) in dichloromethane at room temperature. Subsequent capping with isocyanates, and with α-bromoketones in the presence of polymer-supported Hunigs base (3.0 mmol/g loading, 2.0 equiv),[15] followed by scavenging of excess electrophile with amine resin furnished the desired products. All *N*-capping experiments were conducted using stock solutions of starting materials in acetonitrile at elevated temperature, using a 48-well Flex-Chem block.[16] Consumption of electrophiles was monitored by TLC, and the products **24**–**35** were subsequently isolated by filtration. The presence of the final products, with purities greater than 70%, was confirmed by LCMS.[5]

**Table 1.** Data for examples of *N*-alkyl isatins prepared using parallel synthesis

| Compound | $R^1$ | $R^2$ | $R^3$ | $R^4$ | Isolated purity[7,8] |
|---|---|---|---|---|---|
| **1** | $CH_3$ | H | $CH_3$ | *m*-$OCH_3$-Ph | >90 |
| **2** | H | H | H | $NH_2$ | >90 |
| **3** | H | H | H | *p*-Cl-Ph | >90 |
| **4** | H | H | H | *tert*-Bu | >90 |
| **5** | $CH_3$ | H | $CH_3$ | *p*-Br-Ph | >90 |
| **6** | $CF_3O$ | H | H | 2-Naphthyl | >90 |
| **7** | H | H | H | p-$CH_3$-Ph | >90 |
| **8** | $CH_3$ | H | $CH_3$ | $CH_3$ | >90 |
| **9** | $NO_2$ | H | H | p-$CH_3$-Ph | 40 |
| **10** | H | H | H | *p*-F-Ph | 63 |




**Scheme 2.** Synthesis of piperazine-functionalised isatin analogues using polymer-supported reagents.

**Table 2.** Representative piperazine-functionalised isatin analogues

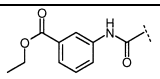
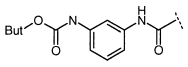
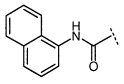
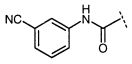
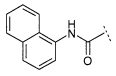
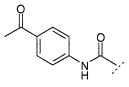
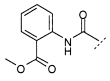
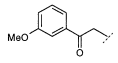
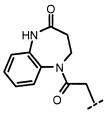
| Compound | $R^1$ | $R^2$ | $R^3$ | Theoretical mass | Mass determined (M + 1) |
|---|---|---|---|---|---|
| **24** | $NO_2$ | H |  | 509.48 | 510.0 |
| **25** | $NO_2$ | H |  | 582.64 | 583.0 |
| **26** | $CF_3O$ | H |  | 526.48 | 527.0 |
| **27** | $CF_3O$ | H |  | 501.43 | 502.0 |
| **28** | Cl | H |  | 476.92 | 477.2 |
| **29** | $CH_3$ | $CH_3$ |  | 462.51 | 463.0 |
| **30** | $CH_3$ | $CH_3$ |  | 426.51 | 427.0 |
| **31** | H | H |  | 450.45 | 450.9 |
| **32** | H | H |  | 421.46 | 422.0 |
| **33** | H | H |  | 343.39 | 344.0 |
| **34** | $CH_3$ | H |  | 357.41 | 358.2 |
| **35** | Cl | H |  | 509.95 | 510.2 |

**Table 3.** Percentage inhibition @ 20 μg/mL[17,18]

| Compound | Human chymotrypsin | Human leukocyte elastase | Human plasmin |
|---|---|---|---|
| **2** | 5 | 3 | 29 |
| **3** | 89 | 56 | 87 |
| **4** | 85 | 51 | 55 |
| **6** | 97 | 64 | 86 |
| **7** | 94 | 45 | 84 |
| **10** | 96 | 90 | 89 |
| **24** | 83 | 95 | 77 |
| **25** | 87 | 94 | 82 |
| **26** | 79 | 79 | 77 |
| **27** | 77 | 26 | 57 |
| **28** | 82 | 74 | 80 |
| **29** | 60 | 97 | 66 |
| **35** | 74 | 79 | 61 |
| Chymostatin (0.5 μg/mL) | 95 | NA | NA |
| BOC-Ala-Ala-Ala-NH-Bz (100 μM) | NA | 72 | NA |
| $\alpha_2$ Antiplasmin (0.5 μg/mL) | NA | NA | 92 |

We then evaluated the biological activity of the compounds against a panel of mammalian serine proteases, including human chymotrypsin, human leukocyte elastase and human plasmin.[17] Biological data for selected compounds is listed in Table 3.[18] As can be seen, good activity was observed in a number of cases, though selectivity between each enzyme target was limited.

In summary we have outlined the convenient, parallel-solution synthesis of isatin analogues using functionalised polymers; several hundred analogues were prepared in this exercise. The supported reagents were employed as stoichiometric reagents, catalysts, and purification media. Activity of these compounds in serine protease assays was observed.

## Acknowledgements

We would like to thank Mrs Andrea Petschner for her assistance in this work, and Dr. Richard Storer and Dr. Terry Bowlin for their comments.

## References and Notes

1. Iyer, R. A.; Hanna, P. E. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 89.
2. Webber, S. E.; Tikhe, J.; Worland, S. T.; Fuhrman, S. A.; Hendrickson, T. F.; Matthews, D. A.; Love, R. A.; Patick, A. K.; Meador, A. W.; Ferre, R. A.; Brown, E. L.; DeLisle, D. M.; Ford, S. E.; Binford, S. L. *J. Med. Chem.* **1996**, *39*, 5072.
3. Available from Fluka.
4. Aminomethyl polystyrene resin (1.0 equiv, 1.2 mmol/g loading) from Novabiochem, or preferably a pre-packed cartridge containing an amine sorbent [bonded silica (aminopropyl functionality) of 0.6 meq/g capacity] from IST Technologies Ltd were both used effectively. Isolation of products in parallel was accomplished using a 20 channel vacuum manifold available from IST Technologies Ltd.
5. Xu, W.; Mohan, R.; Morrissey, M. M. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1089.
6. Habermann, J.; Ley, S. V.; Scott, J. S. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1253.
7. Purities were judged by HPLC-MS at 215 nm and by 400 MHz $^1$H NMR. In several cases, trituration with methyl-*tert*-butyl ether greatly enhanced the levels of purity obtained, and allowed for the products to be isolated as fine powders. We judged the lower purities observed in entries 9 and 10 of Table 1 to be unique cases, perhaps resulting from a degree of experimental error, and not to be representative of the success of the approach as a whole.
8. Data: **1:** $\delta_H$ (400 MHz, DMSO-$d_6$) 7.40 (2H, m), 7.35 (1H, s), 7.20 (1H, d), 6.90 (1H, s), 6.50 (1H, s), 5.10 (2H, s), 3.90 (3H, s), 2.10 (3H, s), 2.05 (3H, s). M + 1 found 324; $C_{19}H_{17}NO_4$ requires 323.35. **2:** $\delta_H$ (400 MHz, DMSO-$d_6$) 7.80 (1H, s), 7.70 (1H, t), 7.60 (1H, d), 7.30 (1H, s), 7.20 (1H, t), 7.00 (1H, d), 4.20 (2H, s). M + 1 found 205; $C_{10}H_8N_2O_3$ requires 204,19. **3:** $\delta_H$ (400 MHz, DMSO-$d_6$) 8.10 (2H, d), 7.65 (2H, d), 7.60 (2H, m), 7.20 (2H, m), 5.40 (2H, s). M + 1 found 300; $C_{16}H_{10}ClNO_3$ requires 299.72. **4:** $\delta_H$ (400 MHz, DMSO-$d_6$) 7.70 (1H, d), 7.55 (1H, t), 7.20 (1H, t), 6.60 (1H, d), 4.80 (1H, s), 1.30 (9H, s). M + 1 found 246; $C_{14}H_{15}NO_3$ requires 245.28. **5:** $\delta_H$ (400 MHz, DMSO-$d_6$) 7.80 (4H, 2d), 7.60 (1H, d), 7.30 (1H, s), 7.00 (1H, d), 5.59 (1H, q), 2.20 (3H, s), 1.45

(2H, d). M + 1 found 373; $C_{18}H_{14}BrNO_3$ requires 372.22. **6:** $\delta_H$ (400 MHz, DMSO-$d_6$) 9.00 (1H, s), 8.20–7.90 (4H, m), 7.80–7.60 (3H, m), 7.40 (1H, d), 7.00 (1H, d), 5.70 (2H, s). M + 1 found 400; $C_{21}H_{12}F_3NO_4$ requires 399.33. **7:** $\delta_H$ (400 MHz, $CDCl_3$) 8.00 (2H, d), 7.75 (1H, d), 7.60 (1H, t), 7.30 (2H, d), 7.15 (1H, t), 6.70 (1H, d), 5.20 (1H, s), 2.45 (3H, s). M + 1 found 280; $C_{17}H_{13}NO_3$ requires 279.30. **8:** $\delta_H$ (400 MHz, $CDCl_3$) 7.55 (1H, s), 7.45 (1H, d), 6.60 (1H, d), 5.00 (1H, q), 2.30 (3H, s), 2.10 (3H, s), 1.55 (3H, d). M + 1 found 231; $C_{13}H_{13}NO_3$ requires 231.25. **9:** $\delta_H$ (400 MHz, $CDCl_3$) 8.50 (1H, s), 8.40 (1H, d), 8.00 (2H, d), 8.40 (2H, d), 6.90 (1H, d), 5.35 (2H, s), 2.20 (3H, s). M + 1 found 325; $C_{17}H_{12}N_2O_5$ requires 324.30. **10:** $\delta_H$ (400 MHz, $CDCl_3$) 8.10 (2H, d), 7.75 (1H, d), 7.60 (1H, t), 7.20 (2H, d), 7.15 (1H, t), 6.70 (1H, d), 5.20 (2H, s). M + 1 found 284; $C_{16}H_{10}NFO_3$ requires 283.26.

9. Edwards, P. D.; Andisik, D. W.; Strimpler, A. M.; Gomes, B.; Tuthill, P. A. *J. Med. Chem.* **1996**, *39*, 1112 and references cited therein.

10. As determined by MACCS II Drug Data Report analysis.

11. Available from Varian Inc.

12. Available from IST Technologies Ltd.

13. Data **11:** $\delta_H$ (400 MHz, $CDCl_3$) 4.05 (2H, s), 3.80–3.60 (8H, m), 1.50 (9H, s). M + 1 found 263; $C_{11}H_{19}ClN_2O_3$ requires 262.74.

14. Data: **12:** $\delta_H$ (400 MHz, $CDCl_3$) 7.70 (1H, d), 7.6 (1H, t), 7.20 (1H, t), 6.95 (1H, br d), 4.60 (2H, s), 3.80–3.40 (8H, m), 1.50 (9H, s). M + 1 found 373; $C_{19}H_{23}N_3O_5$ requires 373.41. **13:** $\delta_H$ (400 MHz, $CDCl_3$) 7.70 (1H, s), 7.6 (1H, d), 6.95 (1H, br d), 4.60 (2H, s), 3.80–3.40 (8H, m), 1.50 (9H, s). M + 1 found 408; $C_{19}H_{22}ClN_3O_5$ requires 407.86. **14:** $\delta_H$ (400 MHz, $CDCl_3$) 8.70 (1H, s), 8.50 (1H, d), 7.10 (1H, br d), 4.10 (2H, s), 3.70–3.40 (8H, m), 1.50 (9H, s). M + 1 found 419; $C_{19}H_{22}N_4O_7$ requires 418.41. **15:** $\delta_H$ (400 MHz, $CDCl_3$) 7.60 (1H, s), 7.10 (1H, d), 7.00 (1H, br d), 4.50 (2H, s), 3.80–3.40 (8H, m), 1.50 (9H, s). M + 1 found 452; $C_{20}H_{22}F_3N_3O_6$ requires 457.41. **16:** $\delta_H$ (400 MHz, $CDCl_3$) 7.40 (1H, s), 7.05 (1H, s), 6.95 (1H, d), 5.10 (2H, s), 3.80–3.40 (8H, m), 2.20 (3H, s), 1.50 (9H, s). M + 1 found 388; $C_{20}H_{25}N_3O_5$ requires 387.44. **17:** $\delta_H$ (400 MHz, $CDCl_3$) 7.35 (1H, s), 7.05 (1H, s), 4.90 (2H, s), 3.80–3.40 (8H, m), 2.30 (3H, s), 2.20 (3H, s), 1.50 (9H, s). M + 1 found 402; $C_{21}H_{27}N_3O_5$ requires 401.47.

15. Available from Argonaut Technologies Inc.

16. Available from Robbins Scientific Inc.

17. Assay protocol: (a) Tris–HCl buffer (50 mM pH 7.2 @ 37 °C); (b) glycerol 15%, incubation time 60 min @ 37 °C ; (c) 100 μL reaction volume, 96-well plate format; (d) fluorescence based assay; monitoring at excitation/emission wavelengths of 370/460 nm.

18. (a) Human chymotrypsin: Sigma # C 8946 (from human pancreas) chymotrypsin in Rx: 800 pM; chymotrypsin substrate: Bachem # I-1465 (Suc-Ala-Ala-Pro-Phe-AMC); substrate in Rx: 50 μM; $K_m$: 33 μM; control inhibitor: Sigma # C 7268 (chymostatin). (b) Human elastase: Sigma # E 8140 (from human leukocytes); elastase in Rx: 40 nM; elastase substrate: Bachem # I-1335 (Suc-Ala-Pro-Ala-AMC); substrate in Rx: 100 μM; $K_m$: 92 μM; control inhibitor: Calbiochem # 324692 (elastase inhibitor). (c) Human plasmin: Enzyme Research Lab. (from human plasma); plasmin in Rx: 165 pM; plasmin substrate: Bachem # I-1045 (H-Ala-Phe-Lys-AMC); substrate in Rx: 25 μM; $K_m$: 19 μM; control inhibitor: Calbiochem # 178221 ($\alpha_2$-antiplasmin). Isatins: dissolved in DMSO @ 10 mg/mL; diluted @ 20 μg/mL in assay. Quenching: fluorescence quenching of compounds evaluated on free AMC in the same buffer as the enzymatic assays.