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Sureshbabu Dadiboyena, Aïcha Arfaoui, Hassen Amri, F. Javier Piedrafita, Adel Nefzi

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Diversity Oriented Synthesis and IKK inhibition of Aminobenzimidazole Tethered quinazoline-2,4-diones, thioxoquinazolin-4-ones, benzodiazepine-2,3,5-triones, isoxazoles and isoxazolines

Sureshbabu Dadiboyena [a], Aïcha Arfaoui [b] , Hassen Amri [b],
F. Javier. Piedrafita [c] and Adel Nefzi [a,*]

[a]Torrey Pines Institute for Molecular Studies, 11350 SW Village Parkway, Port St. Lucie, FL 34987 USA, [b]Laboratory of Selective Organic Chemistry & Biological Activity, Faculty of Science of Tunis, University El Manar, 2092, Tunisia. [c]Torrey Pines Institute for Molecular Studies, General Atomics Court, San Diego, CA 92121 USA,

Abstract: The derivatization of resin-bound aminobenzimidazole toward The parallel solid-phase synthesis of aminobenzimidazole tethered pharmacologically important heterocycles such as quinazoline-2,4-diones, thioxoquinazolin-4-ones, benzodiazepine-2,3,5-triones, isoxazoles and isoxazolines is reported. All the compounds were tested for IKK inhibition. Only one compound elicited significant inhibition of IKKε, TBK-1 and IKK2.

Keywords: Solid-phase synthesis, aminobenzimidazoles, quinazolinediones, thioxoquina-zolinones, benzodiazepinetriones, Intramolecular cyclization, Isoxazoles, Isoxazolines, 1,3-Dipolar cycloaddition, and heterocycles.

Corresponding author:

adeln@tpims.org

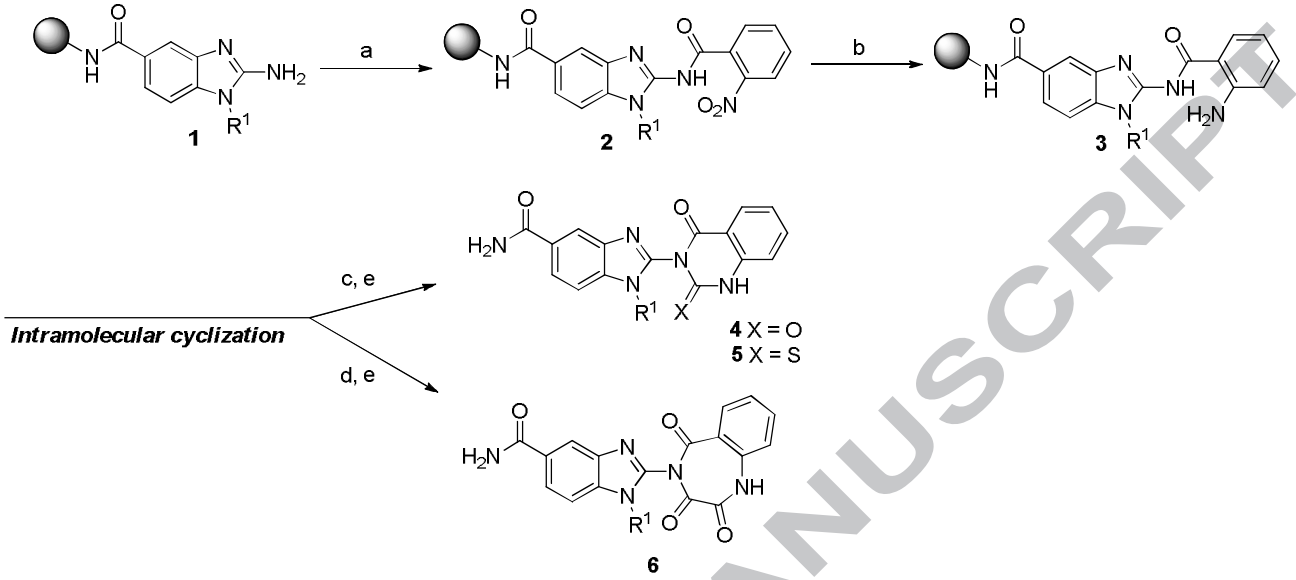
Phone: 1-772-345-4739, Fax: 1-772-345-3649

One of the central objectives of organic and medicinal chemistry is the design, synthesis, and production of molecules having value as human therapeutic agents. Historically, the identification of such compounds has been carried out using compounds from plant and animal tissue extracts, microbial broth extracts, as well as individual compound collections resulting from fifty years of effort by synthetic chemists in academic and pharmaceutical organizations. Diversity-Oriented Synthesis (DOS), is a process by which multiple compounds are generated simultaneously, in a predictable fashion using techniques that involve parallel chemical transformations.[1-4] It allows chemists to achieve more structural complexity than in the early days of combinatorial chemistry.[4-7] Benzimidazoles are an important class of heterocycles displaying a wide array of biological properties,[8-10] and represent a key structural motif in angiotensin-II-antagonists, NMDA antagonists, anticoagulants, and gastric proton-pump inhibitors.[11-14] We previously reported the use of resin-bound aminobenzimidazoles for the synthesis of a variety of fused and/or tethered heterocyclic compounds.[15-19]

Continuing with our interest with the diversification of aminobenzimidazoles, we describe herein a multistep approach for the parallel synthesis of structurally diverse aminobenzimidazole tethered pharmacologically known heterocycles such as quinazoline-2,4-diones, thioxoquinazolin-4-ones, benzodiazepine-2,3,5-triones, isoxazoles and isoxazolines. Substituted quinazolinedione, thioxoquinazolinone and benzodiazepenetrione are found in natural products,[20] and in various drug based p38 kinase inhibitors,[21] aldose reductase inhibitors,[22] 5-HT2C agonist,[23] and CB2 agonists.[24] Recently, quinazolinediones have been developed as typical anti-psychotic agents for treating Schizophrenia and Alzheimer's diseases.[25] Likewise, benzodiazepines are found in several drugs including serotonin and dopamine receptors,[26] 5-HT2C receptors,[27] and glycogen synthase kinase-3 inhibitors.[28]

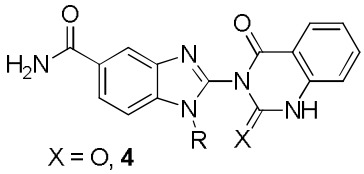
The parallel solid-phase synthesis (tea-bag technology)[29] of aminobenzimidazole tethered quinazoline-2,4-diones, thioxoquinazolin-4-ones and benzodiazepine-2,3,5-triones is outlined in Scheme 1. Resin-bound aminobenzimidazole template **1** [30] was coupled to 2-nitrobenzoic acid in the presence of PyBOP to provide an essential N-benzimidazolylnitrobenzamide precursor **2** which, following tin (II) chloride reduction generated an amine **3**. Treatment of the amine **3** with 1,1'-carbonyldiimidazole generated an isocyanate intermediate which, upon intramolecular cyclization furnished the resin-bound quinazoline-2,4-diones **4**. Similarly, the separate treatment of compound **3** with 1,1'-thiocarbonyldiimidazole and oxalyldiimidazole afforded following thioxoquinazolin-4-ones **5** and benzodiazepine-2,3,5-triones **6**.

The resin was cleaved with HF/anisole and the desired quinazoline-2,4-diones **4**, thioxoquinazolin-4-ones **5**, and benzodiazepine-2,3,5-triones **6** were isolated in moderate yields (30-55%) (Table 1).

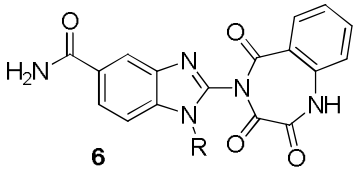


Scheme 1: Synthesis of quinazoline-2,4-dione, thioxoquinazolin-4-one, and benzodiazepine-2,3,5-trione derivatives: (a) 2-nitrobenzoic acid (8 eq., in ahyd. DMF), PyBOP (8 eq.), DIEA (8 eq.), 8h; (b) $SnCl_2*2H_2O$ (10 eq., 1.0M DMF), 24 h; (c) 1,1'-carbonyldiimidazole (or) 1,1'-thiocarbonyldiimidazole (10 eq., 0.5 M in anhyd. DMF), 80 oC, 8h; (d) Oxalyldiimidazole (10 eq., 0.5M in anhydous DMF), 80 ^{o}C, 8h; (e) HF/anisole (99:1), 90 min, 0 ^{o}C

Table 1: Benzimidazole tethered Quinazoline-2,4-diones, thioxoquinazolin-4-ones and benzodiazepine-2,3,5-triones.



X = O, **4**
X = S, **5**



6

Entry	R	Yield[a] (%)
4a	Cyclopentyl	37
4b	*n*-Butyl	40
4c	Cyclohexanemethyl	44
4d	*i*-Butyl	35
4e	3-(trifluoromethyl)benzyl	38
5a	Cyclopentyl	46
5b	*n*-Butyl	42
5c	Cyclohexanemethyl	54
5d	*i*-Butyl	40
5e	3-(trifluoromethyl)benzyl	34
6a	Cyclopentyl	51
6b	*n*-Butyl	48
6c	Cyclohexanemethyl	55
6d	*i*-Butyl	44
6e	3-(trifluoromethyl)benzyl	30

Isolated yields of aminobenzimidazole tethered quinazoline-2,4-diones, thioxoquinazolin-4-ones and benzodiazepine-2,3,5-triones: The products were run on a Vydac column, gradients 5–95% formic acid in ACN in 7 min. [a] The yields are based on the weight of purified products and are relative to the initial loading of the resin.

The application of this solid-phase methodology was explored to a further extent and a series of aminobenzimidazole tethered isoxazoles and isoxazolines were synthesized. We envisioned that aminobenzimidazole coupled alkyne or alkene template would serve as a convenient partner for 1,3-dipolar cycloaddition studies. Recently, we documented the synthesis of an array of isoxazoles and isoxazolines via 1,3-dipolar cycloaddition using resin-bound alkenes and alkynes.[31] In order to build upon this premise, we decided to study the application of aminobenzimidazole based alkyne or alkene template to access a variety of tethered cycloaddition products. Aminobenzimidazole tethered isoxazoles

and isoxazolines were obtained following on resin-bound 1,3-dipolar cycloaddition of aminobenzimidazole acylated with carboxylic acids bearing alkyne or alkene with nitrile oxides. Isoxazoles and isoxazolines are very important class of active compounds. They display antiviral,[32] antitubulin,[33] as well as anti-inflammatory activities.[34,35] Isoxazoline core is a prevalent feature for several spiroisoxazoline natural products [36-41] and isoxazole motif is found in pharmaceutical drugs such as bextra® and parecoxib.[42-46] Due to aforementioned applications, the syntheses of these isoxazole (isoxazoline) based structural units have received greater attention and a few examples representing isoxazoline (**7-12**) and isoxazole (**13,14**) structural motifs are presented in Figure 1.

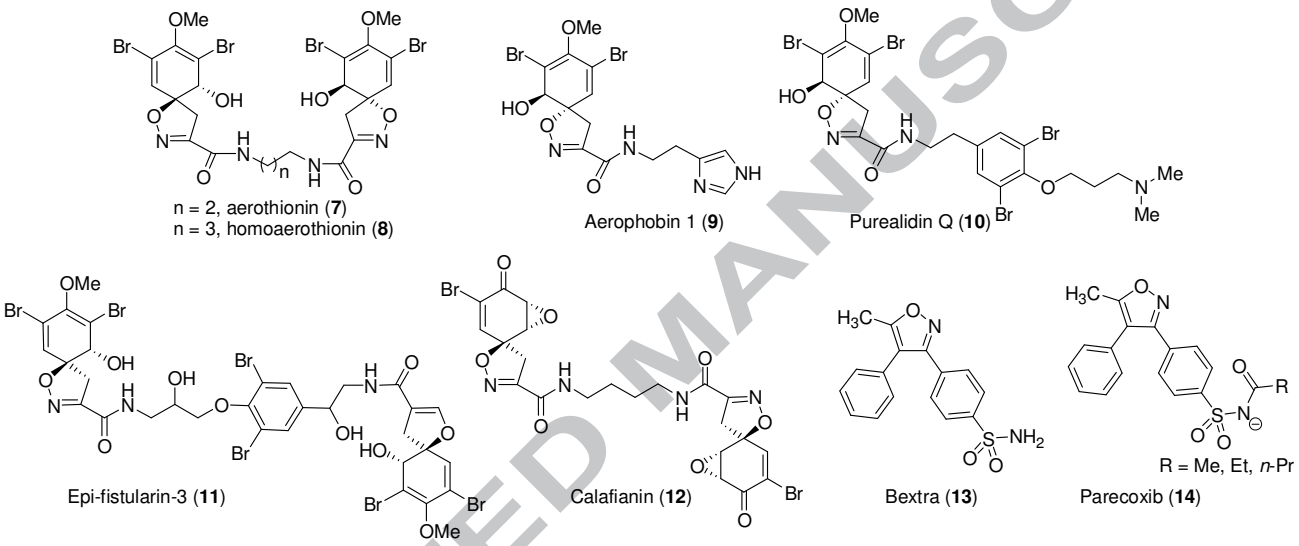
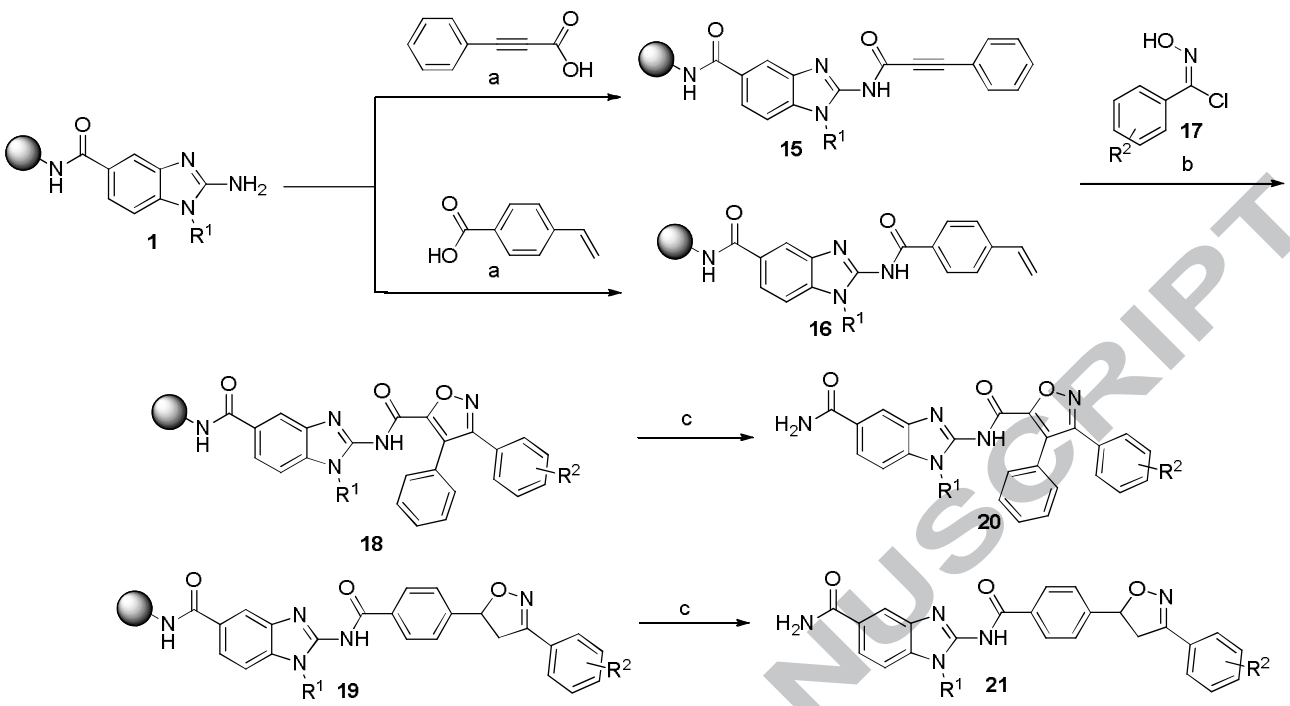


Figure 1: Biologically active isoxazoles and isoxazolines of synthetic and natural origins.

Our approach toward the synthesis of aminobenzimidazole tethered isoxazoles and isoxazolines is outlined in Scheme 2. Following the coupling of phenylpropiolic acid or 4-vinylbenzoic acid to resin-bound aminobenzimidazole 1, the generated alkyne **15** or alkene precursors **16** were treated with freshly prepared hydroximoyl chlorides in the presence of diisopropyldiethylamine (DIEA). The *in situ* formed nitrile oxides reacted with alkenes or alkynes in a 1,3-dipolar fashion[31,47-52] to furnish the corresponding resin-bound isoxazoles **18** or isoxazolines **19**. The cycloaddition reactions on solid-phase occurred with complete regiochemical integrity and the products were isolated in good yields (Table 2). The resin was cleaved with HF/anisole and the desired aminobenzimidazole tethered isoxazoles **20** and racemic isoxazolines **21** were obtained following purification in reasonable yields (12-41%).



Scheme 2: Synthesis of aminobenzimidazole tethered isoxazoles and isoxazolines: (a) Carboxylic acid (8 eq., 0.2M in anhydrous DMF), PyBOP (8 eq.), HOBt (8 eq.), DIEA (8 eq.); (b) hydroximoyl chloride (10 eq., in anhydrous DCM), DIEA (15 eq.) 35 ^{o}C; (c) HF/anisole (95:5), 90 min, 0 ^{o}C.

Table 2: Aminobenzimidazole tethered isoxazoles and isoxazolines

Entry	R^1	R^2	Yield[a] (%)
20a	Cyclopentyl	H	37
20b	*n*-Butyl	H	22
20c	Cyclohexanemethyl	H	33
20d	*i*-Butyl	H	24
20e	3-(trifluoromethyl)benzyl	H	36
20f	Cyclopentyl	Ph	41
20g	*n*-Butyl	Ph	33
20h	Cyclohexanemethyl	Ph	31
20i	*i*-Butyl	Ph	14
20j	3-(trifluoromethyl)benzyl	Ph	28
20k	Cyclopentyl	OMe	25
20l	*n*-Butyl	OMe	12
20m	Cyclohexanemethyl	OMe	40
20n	*i*-Butyl	OMe	15
20o	3-(trifluoromethyl)benzyl	OMe	29
21a	Cyclopentyl	H	41
21b	*n*-Butyl	H	18
21c	Cyclohexanemethyl	H	39
21d	*i*-Butyl	H	14
21e	3-(trifluoromethyl)benzyl	H	35
21f	Cyclopentyl	Ph	32
21g	*n*-Butyl	Ph	30
21h	Cyclohexanemethyl	Ph	41
21i	*i*-Butyl	Ph	17
21j	3-(trifluoromethyl)benzyl	Ph	23
21k	Cyclopentyl	OMe	24
21l	*n*-Butyl	OMe	32
21m	Cyclohexanemethyl	OMe	41
21n	*i*-Butyl	OMe	35
21o	3-(trifluoromethyl)benzyl	OMe	27

Isolated yields of aminobenzimidazole tethered isoxazoles and isoxazolines: The products were run on a Vydac column, gradients 5–95% formic acid in ACN in 7 min. [a] The yields are based on the weight of purified products and are relative to the initial loading of the resin.

We evaluated the effect of all the compounds on the kinase activity of the atypical Inhibitor of kappaB kinases (IKKs), IKK epsilon (IKKε) and TANK-Binding Kinase-1 (TBK-1), [54] which play an essential role in carcinogenesis, inflammation and immunity. In particular, IKKε was identified as a breast proto-oncogene and is overexpressed in many breast cancer cell lines and primary samples. All compounds were first tested at a concentration of 10 μM, and only the Benzimidazole tethered thioxoquinazolin-4-one **5a** elicited significant inhibition (> 50%) of both kinases *in vitro*. The rest of the compounds showed activity less than 10%. A follow up dose response for **5a** was carried out and we included another member of the IKK family, IKK2, as counter screen. We found that compound **5a** was actually a better inhibitor of IKK2 (Figure 2) with an IC_{50} of 2.643 μM. The IC50s for IKKε and TBK-1 were 3.774 and 6.224 μM, respectively.

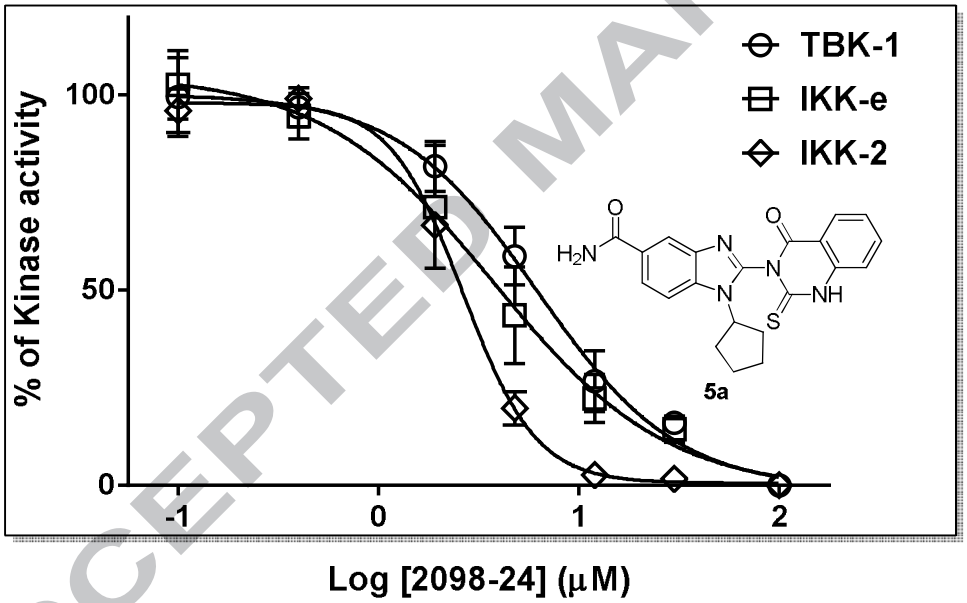


Figure 2: IKK inhibition of Benzimidazole tethered thioxoquinazolin-4-one **5a**

In conclusion, we have developed different multi-step solid-phase strategies for the construction of aminobenzimidazole separately tethered with a variety of biologically important heterocycles, such as quinazoline-2,4-diones, thioxoquinazolin-4-ones, benzodiazepine-2,3,5-triones, isoxazoles and isoxazolines.[52, 53] Following the screening of all the compounds for IKK inhibition, compound **5a** was showed significant inhibition of IKK1, IKKε and IKK2.

Acknowledgments

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References and Notes

1. Schreiber, S. L. Science 2000, 287, 1964.
2. Schreiber, S. L.; Nicolaou, K. C.; and Davies, K. Chem Biol, 2002, 9, 1.
3. Stockwell, B. R. Nature 2004, 432, 846.
4. Thompson, L. A. and Ellman, J. A. Chem. Rev. 1996, 96, 555.
5. Nefzi, A.; Ostresh, J. M.; and Houghten, R. A. Chem. Rev. 1997, 97, 449.
6. Dolle, R. E. J Comb. Chem. 2001, 4, 369.
7. Nicolaou, K. C., Pfefferkorn, J. A., Barluenga, S., Mitchell, H. J., Roecker, A. J., and Cao, G.-Q. J Am Chem Soc. 2000, 122, 9968.
8. Brown, H. D.; Matzuk, A. R.; Ilves, I. R.; Peterson, L. H.; Harris, S. A.; Sarett, L. H.; Egerton, J. R.; Yakstis, J. J.; Campbell, W. C.; Cuckler, A. C. J. Am. Chem. Soc. 1961, 83, 1764.
9. Craigo, W. A.; LeSueur, B. W.; Skibo, E. B. J. Med. Chem. 1999, 42, 3324.
10. Wienen, W.; Hauel, N.; van Meel, J. C.; Narr, B.; Ries, U.; Entzeroth, M. Br J. Pharmacol. 1993, 110, 245.
11. McCanley, J. A.; Munson, P. M.; Thompson, W.; Claremon, D. A. WO0132634, 2000.
12. McCanley, J. A.; Munson, P. M.; Thompson, W.; Claremon, D. A. WO0132634, 2000.
13. Rogers, S. A.; Huigens, R. W. III; Melander, C. J. Am. Chem. Soc. 2009, 131, 9868.
14. Seth, P. A.; Jefferson, E. A.; Risen, L. M.; Osgood, S. A. Bioorg. Med. Chem. Lett. 2003, 13, 1669.
15. Hoesl, C. E.; Nefzi, A.; Houghten, R. A. J. Comb. Chem. 2004, 6, 220
16. Nefzi, A.; Giulianotti, M.; Houghten, R. A. Tetrahedron Lett. 2000, 41, 2283.
17. Dadiboyena, S.; Nefzi, A. Tetrahedron Lett. 2011, 52, 7030.
18. Dadiboyena, S.; Nefzi, A. Synthesis 2012, 44, 215.
19. Dadiboyena, S.; Nefzi, A. Tetrahedron Lett. 2012, 53, 6897.
20. Niwa, K.; Yoshida, Y.; Yamada, K. J. Nat. Prod. 1988, 51, 343.
21. Schlapbach, A.; Heng, R.; Di Padova, F. Bioorg. Med. Chem. Lett. 2004, 14, 357.
22. Malamas, M. S.; Millen, J. J. Med. Chem. 1991, 34, 1492.
23. Ahmad, S.; Ngu, K.; Miller, K. J.; Wu, G.; Hung, C.; Malmstrom, S.; Zhang, G.; O'Tanyi, E.; Keim, W. J.; Cullen, M. J.; Rohrbach, K. W.; Thomas, M.; Ung, T.; Qu, Q.; Gan, J.; Narayanan, R.; Pelleymounter, M. A.; Robl, J. A. Bioorg. Med. Chem. Lett. 2010, 20, 1128.

24. Omura, H.; Kawai, M.; Shima, A.; Iwata, Y.; Ito, F.; Masuda, T.; Ohta, A.; Makita, N.; Omoto, K.; Sugimoto, H.; Kikuchi, A.; Iwata, H.; Ando, K. Bioorg. Med. Chem. Lett. 2008, 18, 3310.
25. Bhat, L.; Mohapatra, P. P.; Dadiboyena, S.; Adiey, K. WO 099503A1, 2010.
26. Guzzo, P. R.; Molino, B. F.; Cui, W.; Liu, S.; Olson, R. E. PTC Int. Appl., 2008141081, 2008.
27. Sabb, A. L.; Vogel, R. L.; Welmaker, G. S.; Sabalski, J. E.; Coupet, J.; Dunlop, J.; Resenzweig-Lipson, S.; Harrison, B. Bioorg. Med. Chem. Lett. 2004, 14, 2603.
28. Magnus, N. A.; Ley, C. P.; Pollock, P. M.; Wepsiec, J. P. Org. Lett. 2010, 12, 3700.
29. Houghten, R. A. Proc. Natl. Acad. Sci. U.S.A. 1985, 82, 5131.
30. Dadiboyena, S.; Nefzi, A. Tetrahedron Lett. 2012, 53, 2096.
31. Lee, Y.-S.; Kim, B. H. Bioorg. Med. Chem. Lett. 2002, 12, 1395.
32. Srivastava, S.; Bajpai, L. K.; Batra, S.; Bhaduri, A. P.; Maikhuri, J. P.; Gupta, G.; Dhar, J. D. Bioorg. Med. Chem. 1999, 7, 2607.
33. Simoni, D.; Grisolia, G.; Giannini, G.; Roberti, M.; Rondanin, R.; Piccagli, L.; Baruchello, R.; Rossi, M.; Romagnoli, R.; Invidiata, F. P.; Grimaudo, S.; Jung, M. K.; Hamel, E.; Gebbia, N.; Crosta, L.; Abbadessa, V.; Di Cristina, A.; Dusonchet, L.; Meli, M.; Tolomeo, M. J. Med. Chem. 2005, 48, 723.
34. Kaffy, J.; Pontikis, R.; Carrez, D.; Croisy, A.; Monneret, C.; Florent, J.-C. Bioorg. Med. Chem. 2006, 14, 4067.
35. Berquist, P. R.; Wells, R. J. Chemotaxonomy of the Porifera: The Development and Current Status of the Field. In Marine Natural Products: Chemical and Biological Perspectives; P. J. Scheuer, Ed.; Academic Press: New York, 1993; Vol. 5, pp 1-50.
36. Faulkner, D. J. Nat. Prod. Rep. 1998, 113.
37. Faulkner, D. J. Nat. Prod. Rep. 1997, 259.
38. Berquist, P. R.; Wells, R. J. In Marine Natural Products; Scheuer, P. J. Ed.; Academic Press: New York, 1983; Vol. V, Chapter 1.
39. Encarnacion, R.D.; Sandoval, E.; Malmstrom, J.; Christophersen, C. J. Nat. Prod. 2000, 63, 874.
40. Dadiboyena, S.; Hamme, A. T. II Tetrahedron Lett. 2011, 52, 2536.
41. Talley, J. J.; Brown, D. L.; Carter, J. S.; Graneto, M. J.; Koboldt, C. M.; Masferrer, J. L.; Perkins, W. E.; Rogers, R. S.; Shaffer, A. F.; Zhang, Y. Y.; Zweifel, B. S.; Seibert, K. J. Med. Chem. 2000, 43, 775.
42. Talley, J. J.; Bertenshaw, S. R.; Brown, D. L.; Carter, J. S.; Graneto, M. J.; Kellogg, M. S.; Koboldt, C. M.; Yuan, J.; Zhang, Y. Y.; Seibert, K. J. Med. Chem. 2000, 43, 1661.
43. Toyokuni, T.; Dileep Kumar, J. S.; Walsh, J. C.; Shapiro, A.; Talley, J. J.; Phelps, M. E.; Herschman, H. R.; Barrio, J. R.; Satyamurthy, N. Bioorg. Med. Chem. Lett. 2005, 15, 4699.
44. Penning, T. D.; Talley, J. J.; Bertenshaw, S. R.; Carter, J. S.; Collins, P. W.; Docter, S.; Graneto, M. J.; Lee, L. F.; Malecha, J. W.; Miyashiro, J. M.; Rogers, R. S.; Rogier, D. J.; Yu,

S. S.; Anderson, G. D.; Burton, E. G.; Cogburn, J. N.; Gregory, S. A.; Koboldt, C. M.; Perkins, W. E.; Seibert, K.; Veenhuizen, A. W.; Zhang, Y. Y.; Isakson, P. C. J. Med. Chem. 1997, 40, 1347.

45. Dadiboyena, S.; Nefzi, A. Eur. J. Med. Chem. 2010, 45, 4697.
46. Dadiboyena, S.; Xu, J.; Hamme, A. T. II Tetrahedron Lett. 2007, 48, 1295.
47. Sandanayaka, V. P.; Youjun, Y. Org. Lett. 2000, 2, 3087.
48. Huisgen, R. Angew. Chem. Intl. Ed. 1963, 2, 565.
49. Huisgen, R. Angew. Chem. Intl. Ed. 1963, 2, 633.
50. Croce, P. D.; La Rosa, C.; Zecchi, G. J. Chem. Soc., Perkin Trans. 1 1985, 2621.
51. Crossley, J. A.; Browne, D. L. J. Org. Chem. 2010, 75, 5414.
52. ***General procedure for the synthesis of amino-benzimidazole tethered quinazoline-2,4-dione, thioxoquinazolin-4-one, and benzodiazepine-2,3,5-triones:*** *p*-Methylbenzhydrylamine (MBHA) resin (100 mg, 1.10 meq/g, 100-200 mesh) was sealed inside a polypropylene mesh packet. Polypropylene bottles were used for all of the reactions. Resin bound aminobenzimidazoles **1** were synthesized according to a previous literature.[15,16] 2-Nitrobenzoic acid (8 equiv.) was coupled to resin bound aminobenzimidazole for 8h using PyBOP (8 equiv.).[17,18] The excess solution was decanted and the resin was washed with DMF (3 times) and DCM (3 times). Reduction of the nitro group was achieved with tin(II) chloride (10 eq., 1.0M in DMF) and the resin was washed with DMF (10 times).[15] Intramolecular cyclizations were performed in a 100 mL glass vial fitted with a screw cap. The N-terminal free amine 3 was treated with 1,1'-carbonyldiimidazole (or) 1,1'-thiocarbonyldiimidazole (or) 1,1'-oxalyldiimidazole (0.5M in anhydrous DMF) and allowed to stir at 80 ^{o}C for 8h.[17] The excess solution was decanted, and the resulting resin-bound aminobenzimidazolyl heterocycles were washed with DMF (2 times) and DCM (2 times). The resin was cleaved with HF/anisole (95:5) for 90 min at 0 ^{o}C and the desired quinazoline-2,4-diones (or) thioxodihydroquinazolin-4-ones (or) benzodiazepine-2,3,5-triones were obtained following extraction with 95% AcOH in H_2O, lyophilized as a colorless powder, and purified by preparative reverse-phase HPLC. **4a**: ^{1}H NMR (DMSO-d_6): δ 1.77-1.81 (m, 2H), 2.07-2.10 (m, 5H), 2.29-2.34 (m, 2H), 5.33 (t, J = 8Hz, 1H), 7.39 (t, J = 8 Hz, 2H), 7.62-7.67 (m, 2H), 7.81 (t, J = 8Hz, 1H), 8.05 (d, J = 8Hz, 1H), 8.13 (br s, 1H), 8.28 (d, J = 8Hz, 1H), 9.05 (s, 1H); LC-MS m/z data calcd. for $C_{21}H_{19}N_5O3$ (M^+): 389.40; found: (MH^+) 390.5; **4b**: ^{1}H NMR (DMSO-d_6): δ 0.93 (t, J = 8 Hz, 4H), 1.33-1.42 (m, 2H), 1.68-1.76 (m, 2H), 3.98 (t, J = 8Hz, 2H), 7.33 (br s, 1H), 7.46 (d, J = 8Hz, 1H), 7.52 (t, J = 8Hz, 1H), 7.76 (d, J = 8Hz, 1H), 7.83-7.88 (m, 2H), 8.05 (br s, 1H), 8.15 (d, J = 8Hz, 1H), 8.81(br s, 1H), 12.43 (br s, 1H); LC-MS m/z data calcd. for $C_{20}H_{19}N_5O_3$ (M^+): 377.40; found (MNa^+): 400.0; **4c**: ^{1}H NMR (DMSO-d_6): δ 0.89-1.07 (m, 6H), 1.47-1.59 (m, 5H), 1.77-1.82 (m, 1H), 3.94 (d, J = 8Hz, 2H), 7.24-7.29 (m, 3H), 7.72-7.78 (m, 2H), 7.90 (d, J = 8Hz, 2H), 7.98 (d, J = 8Hz, 1H), 8.02 (br s, 1H), 8.25 (br s, 1H); LC-MS m/z data calcd. for $C_{23}H_{23}N_5O_3$ (M^+): 417.46; found (MH+): 418.5; **4d**: ^{1}H NMR (DMSO-d_6): δ 0.83 (t, J =

8Hz, 7H), 2.09-2.15 (m, 1H), 3.95 (d, J = 8Hz, 2H), 7.29-7.33 (m, 3H), 7.74-7.82 (m, 2H), 7.91 (d, J = 8Hz, 1H), 8.00 (d, J = 8Hz, 1H), 8.03 (br s, 1H), 8.27 (s, 1H); LC-MS m/z data calcd. for $C_{20}H_{19}N5O3$ (M^+): 377.40; found (MH+): 378.6; **4e**: ^{1}H NMR (DMSO-d_6): δ 5.33 (s, 2H), 7.34 (br s, 1H), 7.40 (d, J = 8Hz, 1H), 7.55 (t, J = 8Hz, 1H), 7.61 (t, J = 8Hz, 1H), 7.68-7.73 (m, 2H), 7.77-7.83 (m, 2H), 7.87-7.91 (m, 3H), 7.99 (br s, 1H), 8.17 (d, J = 8Hz, 1H), 8.82 (s, 1H); LC-MS m/z data calcd. for C24H16F3N5O3 (M^+): 479.41; found (MH+): 480.6; **5a**: ^{1}H NMR (DMSO-d_6): δ 1.59-1.66 (m, 2H), 1.91 (m, 3H), 2.03- 2.09 (m, 2H), 2.11-2.17 (m, 2H), 6.50 (s, 1H), 7.27-7.34 (m, 2H), 7.42 (m, 1H), 7.67 (d, J = 8Hz, 1H), 7.78-7.82 (m, 1H), 7.86 (d, J = 8Hz, 1H), 7.95 (d, J = 8Hz, 1H), 8.01 (br s, 1H), 8.25 (s, 1H); LC-MS m/z data calcd. for C21H19N5O2S (M^+): 405.47; found (MH+): 407.0; **5b**: ^{1}H NMR (DMSO-d_6): δ 0.83 (t, J = 8Hz, 4H), 1.28-1.33 (m, 2H), 1.71-1.76 (m, 2H), 4.12 (t, J = 8Hz, 2H), 7.29 (br s, 1H), 7.42 (t, J = 8Hz, 1H), 7.50 (d, J = 8Hz, 1H), 7.72 (d, J = 8Hz, 1H), 7.85-7.92 (m, 2H), 8.03 (d, J = 8Hz, 2H), 8.25 (br s, 1H); LC-MS m/z data calcd. for $C_{21}H_{19}N_5O_2S$ (M^+): 393.46; found (MH^+): 394.6; **5c**: ^{1}H NMR (DMSO-d_6): δ 0.88-1.08 (m, 6H), 1.48-1.61 (m, 5H), 1.87-1.92 (m, 1H), 3.94-3.97 (m, 2H), 7.29 (br s, 1H), 7.43 (t, J = 8Hz, 1H), 7.51 (d, J = 8Hz, 1H), 7.74 (d, J = 8Hz, 1H), 7.86-7.91 (m, 2H), 8.03-8.05 (m, 2H), 8.25 (s, 1H); LC-MS m/z data calcd. for $C_{23}H_{23}N_5O_2S$ (M^+): 433.52; found (MH^+): 434.6; **5d**: ^{1}H NMR (DMSO-d_6): δ 0.86 (dd, J = 8Hz, 28Hz, 7H), 2.15-2.22 (m, 1H), 3.89-4.00 (m, 2H), 7.29 (br s, 1H), 7.43 (t, J = 8Hz, 1H), 7.51 (d, J = 8Hz, 1H), 7.76 (d, J = 8Hz, 1H), 7.86-7.91 (m, 2H), 8.03 (d, J = 8Hz, 2H), 8.26 (br s, 1H); LC-MS m/z data calcd. for $C_{20}H_{19}N_5O_2S$ (M^+): 393.46; found (MH^+): 394.6; **5e**: 1H NMR (DMSO-d_6): δ 5.56 (s, 2H), 6.50 (s, 1H), 7.29-7.36 (m, 3H), 7.43 (d, J = 8Hz, 1H), 7.51 (t, J = 8Hz, 1H), 7.58 (d, J = 8Hz, 1H), 7.62 (d, J = 8Hz, 1H), 7.71 (s, 1H), 7.79-7.81 (m, 2H), 7.91 (d, J = 8Hz, 1H), 8.00 (br s, 1H), 8.28 (s, 1H); LC-MS m/z data calcd. for $C_{24}H_{16}F_3N_5O_2S$ (M^+): 495.48; found (MH+): 496.4.

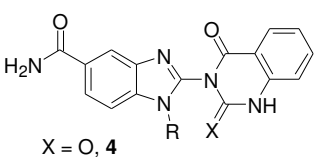
53. ***General procedure for the 1,3-dipolar cycloaddition reaction:*** *p*-Methylbenzhydrylamine (MBHA) resin (100 mg, 1.10 meq/g, 100-200 mesh) was sealed inside a polypropylene mesh packet. Polypropylene bottles were used for all of the reactions. Resin bound amino-benzimidazoles were synthesized according to a previous literature.[15,16] Phenylpropiolic acid (4-vinylbenzoic acid) (8 eq., 0.2M in anhyd. DMF) was coupled to MBHA resin bound amino-benzimidazole for 8 h at room temperature using PyBOP (8 eq.), HOBt (8 eq.) and DIEA (8 eq.) coupling conditions. The excess solution was decanted and the resin was washed with DMF (3 times) and DCM (3 times). The resin-bound amino-benzimidazole tethered alkyne or alkene was treated with a solution of the hydroximoyl chloride (10 eq., 0.2M) in 10 mL of dry DCM and the reaction mixture was stirred overnight at 35°C. The excess solution was decanted, and the resin was washed with DCM (3 times). The resin was cleaved with HF/anisole (95:5) for 90 min at 0°C, and the desired isoxazole (isoxazoline) was obtained following extraction with 95% AcOH in H_2O and lyophilization as a colorless powder. The isoxazole (isoxazoline) was purified by preparative reverse-phase HPLC. **21a**: ^{1}H NMR

(DMSO-d_6): δ 1.75-1.80 (m, 2H), 2.04 (m, 5H), 2.25-2.33 (m, 2H), 3.45 (dd, *J* = 8Hz, 20Hz, 1H), 3.95 (dd, *J* = 8Hz, 20Hz, 1H), 5.35 (m, 1H), 5.80-5.85 (m, 1H), 7.30 (br s, 1H), 7.46-7.55 (m, 7H), 7.71-7.79 (m, 3H), 7.95 (br s, 1H), 8.03 (s, 1H), 8.23 (d, *J* = 8Hz, 2H), 12.88 (br s, 1H); LC-MS *m/z* data calcd. for $C_{29}H_{27}N_5O_3$ (M^+): 493.55; found (MH^+): 495.0; **21b**: ^{1}H NMR (DMSO-d_6): δ 0.93 (t, *J* = 8Hz, 4H), 1.32-1.38 (m, 2H), 1.76-1.83 (m, 2H), 3.45 (dd, *J* = 8Hz, 20Hz, 1H), 3.95 (dd, *J* = 8Hz, 20 Hz, 1H), 4.28 (t, *J* = 8Hz, 2H), 5.80-5.85 (m, 1H), 7.29 (br s, 1H), 7.46-7.51 (m, 4H), 7.55 (d, *J* = 8Hz, 1H), 7.71-7.76 (m, 2H), 7.79 (d, *J* = 8Hz, 1H), 7.95 (br s, 1H), 8.01 (s, 1H), 8.25 (d, *J* = 8Hz, 2H), 12.83 (br s, 1H); LC-MS *m/z* data calcd. for $C_{28}H_{27}N_5O_3$ (M^+): 481.55; found (MH^+): 483.0; **21c**: ^{1}H NMR (DMSO-d_6): δ 1.12-1.16 (m, 5H), 1.58-1.66 (m, 5H), 1.98 (m, 1H), 3.47 (dd, *J* = 8Hz, 20 Hz, 1H), 3.94 (dd, *J* = 8Hz, 20 Hz, 1H), 4.22 (d, *J* = 8Hz, 2H), 5.80-5.85 (m, 1H), 7.29 (br s, 1H), 7.46-7.56 (m, 6H), 7.72-7.77 (m, 2H), 7.78 (d, *J* = 8Hz, 1H), 7.95 (br s, 1H), 8.01 (s, 1H), 8.25 (d, *J* = 8Hz, 2H), 12.81 (br s, 1H); LC-MS *m/z* data calcd. for $C_{31}H_{31}N_5O_3$ (M^+): 521.61; found (MH^+): 523.0; **21d**: ^{1}H NMR (DMSO-d_6): δ 0.95 (d, *J* = 8Hz, 7H), 2.28-2.35 (m, 1H), 3.45 (dd, *J* = 8Hz, 20Hz, 1H), 3.95 (dd, *J* = 8Hz, 20Hz, 1H), 4.09 (d, *J* = 8Hz, 2H), 5.80-5.85 (m, 1H), 7.29 (br s, 1H), 7.46-7.51 (m, 4H), 7.56 (d, *J* = 8Hz, 1H), 7.71-7.78 (m, 2H), 7.79 (d, *J* = 8Hz, 1H), 7.96 (br s, 1H), 8.02 (s, 1H), 8.25 (d, *J* = 8Hz, 2H), 12.81 (br s, 1H); LC-MS *m/z* data calcd. for $C_{28}H_{27}N_5O_3$ (M^+): 481.55; found (MH^+): 483.0; **21e**: ^{1}H NMR (DMSO-d_6): δ 3.44 (dd, *J* = 8Hz, 20Hz, 1H), 3.94 (dd, *J* = 8Hz, 20Hz, 1H), 5.60 (s, 2H), 5.80-5.85 (m, 1H), 7.30 (br s, 1H), 7.46-7.49 (m, 5H), 7.56-7.61 (m, 2H), 7.64-7.78 (m, 5H), 7.94 (br s, 1H), 7.96 (s, 1H), 8.02 (s, 1H), 8.25 (d, *J* = 8Hz, 2H), 12.88 (br s, 1H); LC-MS *m/z* data calcd. for $C_{32}H_{24}F_3N_5O_3$ (M^+): 583.56; found (MH^+): 585.0; **21f**: ^{1}H NMR (DMSO-d_6): δ 1.75-1.77 (m, 2H), 2.05 (m, 4H), 2.27-2.33 (m, 2H), 3.45 (dd, *J* = 8Hz, 20Hz, 1H), 3.98 (dd, *J* = 8Hz, 20Hz, 1H), 5.33-5.35 (m, 1H), 5.83-5.87 (m, 1H), 7.29 (br s, 1H), 7.41 (t, *J* = 8Hz, 1H), 7.48-7.55 (m, 5H), 7.72-7.84 (m, 7H), 7.95 (br s, 1H), 8.03 (s, 1H), 8.23 (d, *J* = 8Hz, 2H), 12.90 (br s, 1H); LC-MS *m/z* data calcd. for $C_{35}H_{31}N_5O_3$ (MH^+): 569.65; found (MH^+): 571.0; **21g**: ^{1}H NMR (DMSO-d_6): δ 0.93 (t, *J* = 8Hz, 4H), 1.30-1.40 (m, 2H), 1.76-1.83 (m, 2H), 3.50 (dd, *J* = 8Hz, 20Hz, 1H), 3.98 (dd, *J* = 8Hz, 20Hz, 1H), 4.29 (t, *J* = 8Hz, 2H), 5.82-5.87 (m, 1H), 7.29 (br s, 1H), 7.38-7.43 (m, 1H), 7.48-7.56 (m, 4H), 7.73 (d, *J* = 8Hz, 2H), 7.77 (m, 5H), 7.95 (br s, 1H), 8.02 (s, 1H), 8.26 (d, *J* = 8Hz, 2H), 12.81 (br s, 1H); LC-MS *m/z* data calcd. for $C_{34}H_{31}N_5O_3$ (M^+): 557.65; found (MH^+): 559.0; **21h**: ^{1}H NMR (DMSO-d_6): δ 1.13-1.16 (m, 5H), 1.58-1.66 (m, 5H), 1.98 (m, 1H), 3.51 (dd, *J* = 8Hz, 20Hz, 1H), 3.98 (dd, *J* = 8Hz, 20Hz, 1H), 4.13 (d, *J* = 8Hz, 2H), 5.83-5.87 (m, 1H), 7.29 (br s, 1H), 7.38-7.43 (m, 1H), 7.48-7.53 (m, 5H), 7.73 (d, *J* = 8Hz, 2H), 7.77-7.84 (m, 5H), 7.95 (br s, 1H), 8.01 (s, 1H), 8.26 (d, *J* = 8Hz, 2H), 12.81 (br s, 1H); LC-MS *m/z* data calcd. for $C_{37}H_{35}N_5O_3$ (M^+): 597.71; found (MH^+): 599.0; **21i**: ^{1}H NMR (DMSO-d_6): δ 0.92 (d, *J* = 8Hz, 7H), 2.29-2.35 (m, 1H), 3.50 (dd, *J* = 8Hz, 20Hz, 1H), 3.98 (dd, *J* = 8Hz, 20Hz, 1H), 4.10 (d, *J* = 8Hz, 2H), 5.83-5.87 (m, 1H), 7.30 (br s, 1H), 7.38-7.43 (m, 1H), 7.48-7.53 (m, 3H), 7.57 (d, *J* = 8Hz, 1H), 7.73 (d, *J* = 8Hz, 2H), 7.77-7.84 (m, 5H), 7.95 (s,

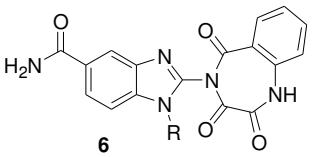
1H), 8.02 (s, 1H), 8.26 (d, *J* = 8Hz, 2H), 12.83 (br s, 1H); LC-MS *m/z* data calcd. for $C_{31}H_{31}N_5O_3$ (M^+): 557.64; found (MH^+): 559.0; **21j**: ^{1}H NMR (DMSO-d_6): δ 3.48 (dd, *J* = 8Hz, 20Hz, 1H), 3.98 (dd, *J* = 8Hz, 20Hz, 1H), 5.62 (s, 2H), 5.83-5.87 (m, 1H), 7.31 (br s, 1H), 7.38-7.43 (m, 1H), 7.48-7.51 (m, 4H), 7.55-7.61 (m, 2H), 7.64-7.74 (m, 5H), 7.76-7.83 (m, 4H), 7.94 (br s, 1H), 7.96 (s, 1H), 8.02 (s, 1H), 8.25 (d, *J* = 8Hz, 2H), 12.90 (br s, 1H); LC-MS *m/z* data calcd. for $C_{38}H_{28}F_3N_5O_3$ (M^+): 659.66; found (MH^+): 661.0; **21k**: ^{1}H NMR (DMSO-d_6): δ 1.75-1.78 (m, 2H), 2.05 (m, 4H), 2.25-2.30 (m, 2H), 3.45 (dd, *J* = 8Hz, 20Hz, 1H), 3.87-3.94 (m, 5H), 5.35 (m, 1H), 5.78-5.83 (m, 1H), 7.25 (d, *J* = 8Hz, 1H), 7.30 (br s, 1H), 7.49 (d, *J* = 8Hz, 2H), 7.54 (d, *J* = 8Hz, 1H), 7.68 (d, *J* = 8Hz, 1H), 7.76-7.79 (m, 2H), 7.96 (br s, 1H), 8.04 (s, 1H), 8.22 (d, *J* = 8Hz, 2H), 12.86 (br s, 1H); LC-MS *m/z* data calcd. for $C_{30}H_{29}N_5O_4$ (M^+): 523.58; found (MH^+): 525.0; **21l**: ^{1}H NMR (DMSO-d_6): δ 0.93 (t, *J* = 8Hz, 4H), 1.30-1.40- (m, 2H), 1.76-1.83 (m, 2H), 3.45 (dd, *J* = 8Hz, 20Hz, 1H), 3.87-3.94 (m, 4H), 4.28 (t, *J* = 8Hz, 2H), 5.78-5.83 (m, 1H), 7.25 (d, *J* = 8Hz, 1H), 7.29 (br s, 1H), 7.49 (d, *J* = 8Hz, 2H), 7.56 (d, *J* = 8Hz, 1H), 7.68 (d, *J* = 8Hz, 1H), 7.77-7.81 (m, 2H), 7.95 (br s, 1H), 8.01 (s, 1H), 8.25 (d, *J* = 8Hz, 2H), 12.79 (br s, 1H); LC-MS *m/z* data calcd. for $C_{29}H_{29}N_5O_4$ (M^+): 511.57; found (MH^+): 513.0; **21n**: ^{1}H NMR (DMSO-d_6): δ 0.96 (d, *J* = 4Hz, 7H), 2.28-2.35 (m, 1H), 3.45 (dd, *J* = 8Hz, 20Hz, 1H), 3.87-3.94 (m, 4H), 4.09 (d, *J* = 8Hz, 2H), 5.78-5.83 (m, 1H), 7.25 (d, *J* = 8Hz, 1H), 7.29 (br s, 1H), 7.49 (d, *J* = 8Hz, 2H), 7.57 (d, *J* = 8Hz, 1H), 7.68 (d, *J* = 8Hz, 1H), 7.77-7.80 (m, 2H), 7.95 (br s, 1H), 8.01 (s, 1H), 8.24 (d, *J* = 8Hz, 2H), 12.86 (br s, 1H); LC-MS *m/z* data calcd. for $C_{29}H_{29}N_5O_4$ (M^+): 511.57; found (MH^+): 513.0; **21o**: ^{1}H NMR (DMSO-d_6): δ 3.45 (dd, *J* = 8Hz, 20Hz, 1H), 3.87-3.94 (m, 3H), 5.61 (s, 2H), 5.78-5.83 (m, 1H), 7.25 (d, *J* = 8Hz, 1H), 7.30 (br s, 1H), 7.47 (d, *J* = 8Hz, 2H), 7.54-7.60 (m, 2H), 7.64-7.70 (m, 4H), 7.75-7.78 (m, 3H), 7.93 (br s, 1H), 7.96 (s, 1H), 8.02 (s, 1H), 8.25 (d, *J* = 8Hz, 2H), 12.90 (br s, 1H); LC-MS *m/z* data calcd. for $C_{33}H_{26}F_3N_5O_4$ (M^+): 613.58; found (MH^+): 615.0.

54. **Kinase assays:** Kinase activity was measured using a LANCE Ultra time-resolved fluorescence resonance energy transfer (TR-FRET) assay and purified recombinant IKKs (Carna Biosciences). Kinases were diluted in kinase buffer (50 mM Hepes pH 7.4, 10 mM $MgCl_2$, 1 mM EGTA, 2 mM DTT, and 0.01% Tween-20) to a final concentration of 2 nM (IKKε), 4 nM (TBK-1), or 1 nM (IKK2). 50 nM Ulight-rpS6 and Ulight-IκBα (Perkin Elmer) were used as peptide substrates for IKKε/TBK-1 and IKK2, respectively. All assays were performed with an ATP concentration close to the apparent *Km* for each enzyme (5 μM for IKKε, 10 μM for TBK-1 and 1.25 μM for IKK2). After 1 h (IKK2) or 2 h incubation (IKKε/TBK-1) at room temperature, the reaction was stopped by addition of 20 mM EDTA in LANCE detection buffer, containing 2 nM Europium-labelled phospho-specific antibody (Perkin Elmer). Two hours later, the TR-FRET signals at 620 and 665 nm were measured in a

CLARIOstar (BMGLabtech) multilabel reader. 665/620 ratios and delta F values were calculated with MARS data analysis program and the IC_{50} values for active compounds were determined using a 7 point titration experiment with GraphPrism.



X = O, **4**
X = S, **5**



6

Entry	R^1	Yield (%)[a]
4a	Cyclopentyl	37
4b	n-Butyl	40
4c	Cyclohexanemethyl	44
4d	i-Butyl	35
4e	3-(trifluoromethyl)benzyl	38
5a	Cyclopentyl	46
5b	n-Butyl	42
5c	Cyclohexanemethyl	54
5d	i-Butyl	40
5e	3-(trifluoromethyl)benzyl	34
6a	Cyclopentyl	51
6b	n-Butyl	48
6c	Cyclohexanemethyl	55
6d	i-Butyl	44
6e	3-(trifluoromethyl)benzyl	30

[a] Yield is based….

[Insert Figure/ scheme Here]

Entry	R^1	R^2	Yield (%)[a]
20a	Cyclopentyl	H	37
20b	n-Butyl	H	22
20c	Cyclohexanemethyl	H	33
20d	i-Butyl	H	24
20e	3-(trifluoromethyl)benzyl	H	36
20f	Cyclopentyl	Ph	41
20g	n-Butyl	Ph	33
20h	Cyclohexanemethyl	Ph	31
20i	i-Butyl	Ph	14
20j	3-(trifluoromethyl)benzyl	Ph	28
20k	Cyclopentyl	OMe	25
20l	n-Butyl	OMe	12
20m	Cyclohexanemethyl	OMe	40
20n	i-Butyl	OMe	15
20o	3-(trifluoromethyl)benzyl	OMe	29
21a	Cyclopentyl	H	41
21b	n-Butyl	H	18
21c	Cyclohexanemethyl	H	39
21d	i-Butyl	H	14
21e	3-(trifluoromethyl)benzyl	H	35
21f	Cyclopentyl	Ph	32
21g	n-Butyl	Ph	30
21h	Cyclohexanemethyl	Ph	41
21i	i-Butyl	Ph	17
21j	3-(trifluoromethyl)benzyl	Ph	23
21k	Cyclopentyl	OMe	24
21l	n-Butyl	OMe	32
21m	Cyclohexanemethyl	OMe	41
21n	i-Butyl	OMe	35
21o	3-(trifluoromethyl)benzyl	OMe	27

[a] Yield is based….

Graphical abstract