Bioorganic & Medicinal Chemistry Letters 22 (2012) 5272–5278

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Eco-friendly synthesis and biological evaluation of substituted pyrano[2,3-*c*]pyrazoles

Santhosh Reddy Mandha [a], Sravanthi Siliveri [b], Manjula Alla [a,*], Vittal Rao Bommena [a], Madhava Reddy Bommineni [b], Sridhar Balasubramanian [c]

[a] *Crop Protection Chemicals Division, Indian Institute of Chemical Technology, Tarnaka, Hyderabad 500607, India*
[b] *Department of Pharmaceutical Chemistry, G. Pulla Reddy College of Pharmacy, Mehdipatnam, Hyderabad 500028, India*
[c] *Laboratory of X-ray Crystallography, Indian Institute of Chemical Technology, Hyderabad 500607, India*

ARTICLE INFO

Article history:
Received 22 February 2012
Revised 31 May 2012
Accepted 15 June 2012
Available online 29 June 2012

Keywords:
Multicomponent reactions
Non-catalytic conditions
Antibacterial activity
Anti-inflammatory activity
Cytotoxic activity

ABSTRACT

An ecofriendly green approach for synthesis of substituted pyrano[2,3-*c*]pyrazoles has been developed via a multicomponent one pot approach in aqueous ethanol medium under totally non-catalytic conditions. The synthesized compounds were evaluated for their antibacterial, anti-inflammatory and cytotoxic activities.

Substituted pyrano[2,3-*c*]pyrazoles are much sought after class of heterocycles because of their potential applications in pharmaceutical field. They exhibit a wide range of biological activities like antimicrobial,[1] anticancer,[2,3] anti-inflammatory,[4] inhibitors of human Chk1 kinase[5] and also as biodegradable agrochemicals.[6] Furthermore, they play a significant role as crucial synthetic intermediates.[7] Synthetic strategies of substituted pyrano[2,3-*c*]pyrazoles have evolved a long way from the earlier reported multistep protocols[8,9] to the present day multi-component reactions (MCR's). Conventional synthetic methodologies reported, though have a broad scope but generate copious amount of waste. As a result chemical industry is subjected to immense pressure to get rid of such waste. In this aspect, multi-component reactions have emerged as a valuable synthetic tool in the perspective of modern drug discovery because of their efficiency, intrinsic atom economy and structural diversity of resulting products, when compared to conventional multistep synthesis.

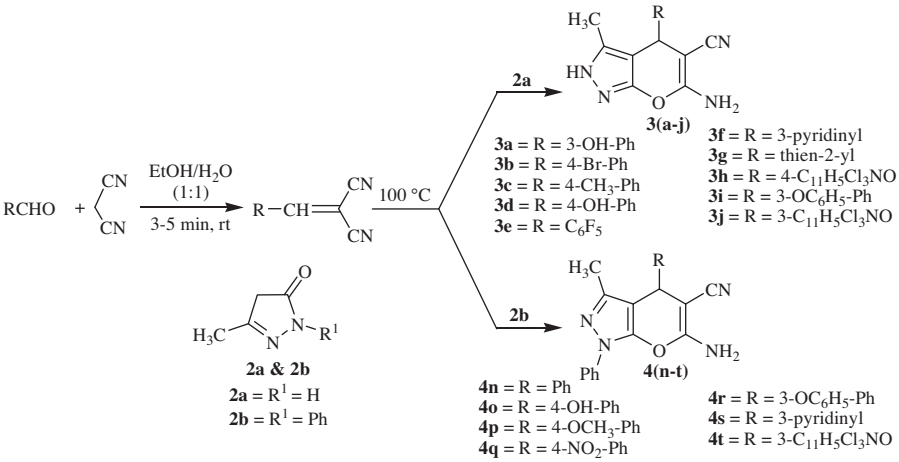
Literature survey reveals a variety of three and four component MCR's for the construction of different pyranopyrazoles. Among them a three-component reaction between aromatic aldehyde, malononitrile, and substituted pyrazolin-5-ones in ethanol medium was reported using triethylamine as catalyst.[10] Subsequently, many synthetic methods have been developed involving either ethanol or water as a solvent with a variety of catalysts.[11] The only non-catalytic approach[12] reported for these compounds was low yielding and thus limiting its scope. Even though there are many non-catalytic methods available in literature for the construction of pyranopyrazoles, construction of N_1-phenylsubstituted pyranopyrazoles are still carried out in a catalytic medium.[13] Herein, we report a simple non-catalytic method for synthesis of both pyranopyrazoles and N_1-phenylsubstituted pyranopyrazoles by using the mixture of water and ethanol (1:1) as a solvent media. Further this protocol is amenable for constructing the spiroindoline and spirofluorene substituted pyranopyrazoles.

At the outset, synthesis of pyrano[2,3-*c*]pyrazoles via a multicomponent approach under non-catalytic conditions as shown in Scheme 1, was envisaged. A one pot four component reaction was carried out with ethylacetoacetate, hydrazine hydrate, malononitrile and 4-hydroxybenzaldehyde in aqueous ethanol medium (1:1) and heated to 100 °C. The reaction was completed in 2.5 h precipitating the desired product **3d** in 80% yield. The one pot four component reactions were attempted with various aryl, heteroaryl aldehydes and chloropyridinyloxy benzaldehydes.[14] Under the optimized conditions, irrespective of the substituent present on the aromatic ring containing aldehyde moiety, corresponding were obtained in good yields. The substituent patterns are given in Table 1.

However, when the one pot reaction was attempted by replacing one of the substrates that is, hydrazine hydrate with phenyl

* Corresponding author. Tel.: +91 040 27191441.
E-mail address: manjula@iict.res.in (M. Alla).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.06.055



Scheme 1. Construction of various substituted pyrano[2,3-*c*]pyrazoles.

Table 1
Yields and melting point values of pyranopyrazole derivatives

Entry	R	R^1	Product	Time (h)	Yield[a] (%)	Mp[b] (°C)
1	3-OH–C_6H_4	H	**3a**	2.2	75	248–249
2	4-Br–C_6H_4	H	**3b**	2.5	80	178–179[15]
3	4-CH_3–C_6H_4	H	**3c**	3.0	84	206–207[15]
4	4-OH–C_6H_4	H	**3d**	2.5	80	225–227[15]
5	C_6F_5	H	**3e**	2.5	64	249–250
6	3-C_5H_4N	H	**3f**	3.0	88	224–226
7	2-C_4H_3S	H	**3g**	3.0	80	223–225
8	4-$C_{11}H_5Cl_3NO$	H	**3h**	3.0	87	236–237
9	3-OC_6H_5–C_6H_4	H	**3i**	2.5	87	203–204
10	3-$C_{11}H_5Cl_3NO$	H	**3j**	2.5	88	224–225
11	—	H	**3k**	2.5	89	274–276[16]
12	—	H	**3l**	3.0	86	244–247
13	—	H	**3m**	3.0	88	248–250
14	C_6H_5	C_6H_5	**4n**	2.5	88	169–171[17]
15	4-OH–C_6H_4	C_6H_5	**4o**	2.5	86	210–212[18]
16	4-OCH_3–C_6H_4	C_6H_5	**4p**	3.0	85	174–175[17]
17	4-NO_2–C_6H_4	C_6H_5	**4q**	1.5	80	195–197[17]
18	3-OC_6H_5–C_6H_4	C_6H_5	**4r**	2.0	86	202–203
19	3-C_5H_4N	C_6H_5	**4s**	2.5	93	213–215[19]
20	3-$C_{11}H_5Cl_3NO$	C_6H_5	**4t**	3.0	89	137–139
21	—	C_6H_5	**4u**	2.0	80	219–221[16]
22	—	C_6H_5	**4v**	2.5	81	198–199

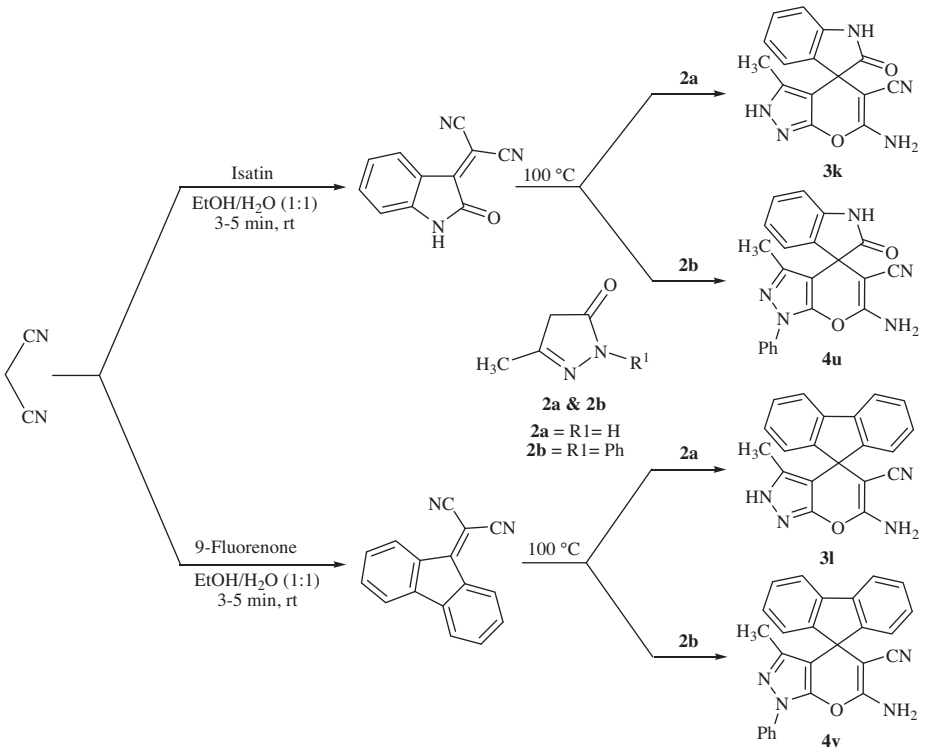
[a] Isolated yields.
[b] Literature references.

hydrazine, reaction was too sluggish. In order to improve the rate of reaction, 3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (**2b**) was isolated by performing an initial condensation reaction between ethylacetoacetate and phenyl hydrazine through a known procedure.[20] This was followed by the addition of carbonyl compound and malononitrile to 3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (**2b**) in aqueous ethanol medium to give the products **4n**–**v** in excellent yields. Likewise, the isolation of 3-methyl-1H-pyrazol-5(4H)-one (**2a**) and reacting it with carbonyl compound and malononitrile in aqueous ethanol medium resulted in better yields for the synthesis of compounds **3a**–**m** when compared to corresponding one pot four component reaction. Another salient feature of this protocol is that the reaction is facile with the ketones that is, isatin and 9-fluorenone (Table 1, entry 11–12 and 21–22) and a dialdehyde terephthalaldehyde (Table 1, entry 13) as shown in Schemes 2 and 3.

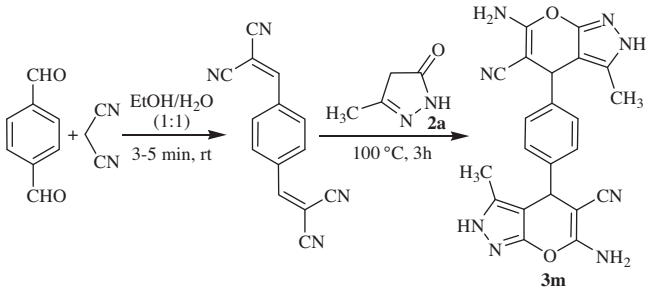
The mechanism of reaction is believed to involve the Knoevenagel condensation[21] with aromatic carbonyl compounds and malononitrile to form an alkene intermediate in situ. The intermediate undergoes Michael addition with pyrazolin-5-one (formed in situ from ethylacetoacetate and hydrazine hydrate) followed by an intra-molecular cyclization to give polyfunctionalized pyrano [2,3-*c*]pyrazoles. Knoevenagel condensation is known to occur in non-catalytic conditions in protic solvents[22] and could well be the driving force for the non-catalytic transformation. The structures of newly synthesized compounds were assigned on the basis of their spectral data and those reported compounds by comparing with earlier literature.

X-ray studies and molecular structure of pyranopyrazole (**3e**) revealed that it exists in the crystal phase as a 2H, instead of 1H-tautomer as shown in Figure 1(a), which is in congruence with the literature reports on pyranopyrazoles.[23] In contrast to this, N_1-substituted pyranopyrazoles exist as 'immobilized' 1H-tautomers.[24] X-ray structure of the compound **3e**[25] was shown in Figure 1(b).

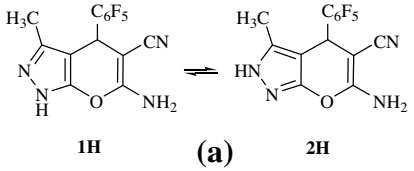
Antibacterial studies: All the synthesized compounds were screened for their antibacterial activity against two Gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*), two Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) with ciprofloxacin as a standard. The study was carried out by Cup-plate



Scheme 2. Construction of spiroindoline and spirofluorene substituted pyrano[2,3-*c*]pyrazoles.



Scheme 3. Construction of 4,4′-(1,4-phenylene)bis(6-amino-3-methyl-1,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitrile).



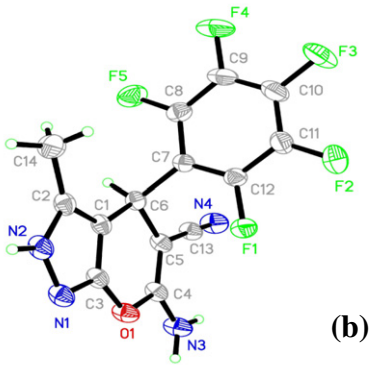


Figure 1. (a) 1H, 2H-tautomers of **3e**. (b) The molecular structure of **3e**, with the atom-numbering scheme. Displacement ellipsoids are drawn at the 30% probability level and H atoms are shown as small spheres of arbitrary radius.

method[26] to determine the zone of inhibition (mm) against four strains of bacteria. Antibacterial activity has been carried out at a concentration of 100 μg/50 μL. Investigation of antibacterial data revealed that compounds **3e**, **3g**, **3h**, **3j**, **4o**, **4t** and **4u** showed good activity against all the bacterial strains and **4r** showed good activity against two strains *Staphylococcus aureus* and *Escherichia coli* as shown in Table 2.

Screening of the compounds **3e**, **3g**, **3h**, **3j**, **4o**, **4r**, **4t** and **4u** for their antibacterial activity were performed at different concentrations that is, 0.39, 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50 and 100 μg/mL by using Broth Microdilution MIC method. All the compounds showed antibacterial activity against both gram positive and gram negative standard strains and their MICs ranged between 1.56 and 12.5 μg/mL. The MICs of these compounds and ciprofloxacin were determined by using the standard protocol of NCCLS Broth Microdilution MIC method[27] and the results are tabulated in Table 3.

Anti-inflammatory studies: The inflammatory activities of some of the synthesized compounds were evaluated using the Carrageenan induced paw edema bioassay in rats[28] shown in Table 4.

The results revealed that of all the tested compounds **3g–k**, **4u** and **4v**, exhibited significant anti-inflammatory activity (% protection 64.5, 72.0, 66.6, 70.9, 69.8, 68.8, 65.5, respectively) comparable to that of standard Ibuprofen (% protection 72.3).

By using mean paw edema values percentage protection against edema formation calculated (Table 5) and also the percentage protection of pyranopyrazole derivatives against edema formation are represented in the form of graph as shown in Figure 2.

Cytotoxic activity evaluation (in vivo studies): Evaluation of cytotoxic activity of the synthesized compounds against MCF-7 (breast cancer cell line) by MTT assay with Taxol as a standard reference[29] has been carried out and the results are presented in Table 6.

Table 2
Antibacterial data of pyrano[2,3-*c*]pyrazoles

Compound	Concentration (μg/50μL)	Zone of inhibition (mm)			
		S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
Solvent (control)	—	—	—	—	—
Ciproflo-xacin	25	20	28	30	20
3a	100	8	—	—	—
3b	100	—	—	—	—
3c	100	10	10	—	15
3d	100	13	—	15	15
3e	100	18	15	14	15
3f	100	—	—	—	—
3g	100	15	18	20	14
3h	100	15	20	22	14
3i	100	7	9	9	11
3j	100	16	21	23	15
3k	100	13	11	12	10
3l	100	10	10	10	17
3m	100	—	—	—	—
4n	100	6	8	14	9
4o	100	15	17	16	15
4p	100	5	4	10	6
4q	100	8	7	15	8
4r	100	15	—	18	—
4s	100	—	—	—	—
4t	100	16	15	14	16
4u	100	14	19	21	15
4v	100	12	11	13	16

Table 3
Antibacterial activity of different compounds by Broth Microdilution MIC method

Concn (μg/mL)	Antibacterial activity against standard strains—compounds																													
	3e				**3g**				**3h**				**3j**				**4o**				**4r**				**4t**				**4u**			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0.39	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.78	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1.56	+	+	–	–	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.125	–	–	–	–	–	–	–	–	+	–	–	–	+	+	–	+	+	+	+	–	+	–	+	–	+	+	+	+	–	–	–	+
6.25	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+	–	–	–	–	–
12.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
25	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
50	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
100	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

1, *Bacillus subtilis*; 2, *Staphylococcus aureus*; *3*, *Escherichia coli*; 4, *Pseudomonas aeruginosa*; +, resistant; –, susceptible.
Ciprofloxacin is taken as a standard drug and its MIC is 1.56 μg/mL against all the four strains.

Table 4
Anti-inflammatory activity data of pyrano[2,3-*c*]pyrazoles

Treatment	Dose (mg/kg)	Mean paw edema volume in mL ± SEM[a,b]				
		30 min	1 h	2 h	3 h	4 h
Control	100	0.17 ± 0.004	0.36 ± 0.003	0.73 ± 0.003	0.93 ± 0.004	0.91 ± 0.003
Standard (Ibuprofen)	100	0.11 ± 0.003	0.22 ± 0.004	0.33 ± 0.003	0.25 ± 0.003	0.33 ± 0.003
3g	100	0.13 ± 0.005	0.22 ± 0.008	0.36 ± 0.004	0.33 ± 0.012	0.44 ± 0.011
3h	100	0.12 ± 0.003	0.18 ± 0.005	0.24 ± 0.003	0.26 ± 0.003	0.41 ± 0.003
3i	100	0.14 ± 0.003	0.24 ± 0.004	0.38 ± 0.005	0.31 ± 0.00	0.35 ± 0.003
3j	100	0.13 ± 0.003	0.19 ± 0.005	0.26 ± 0.003	0.27 ± 0.003	0.42 ± 0.004
3k	100	0.13 ± 0.003	0.23 ± 0.004	0.30 ± 0.003	0.28 ± 0.003	0.44 ± 0.011
4n	100	0.15 ± 0.002	0.26 ± 0.003	0.42 ± 0.002	0.43 ± 0.009	0.47 ± 0.011
4o	100	0.15 ± 0.002	0.25 ± 0.012	0.41 ± 0.003	0.40 ± 0.012	0.45 ± 0.008
4p	100	0.15 ± 0.004	0.27 ± 0.008	0.44 ± 0.005	0.44± 0.004	0.50 ± 0.008
4u	100	0.13 ± 0.011	0.27 ± 0.003	0.39 ± 0.008	0.29 ± 0.004	0.48 ± 0.012
4v	100	0.13 ± 0.005	0.25 ± 0.003	0.40 ± 0.004	0.32 ± 0.011	0.40 ± 0.005

[a] SEM denotes Standard Error of Mean.
[b] All data are significantly different from control ($P < 0.001$).

All tested compounds have shown significant cytotoxicity. Compounds **3e**, **3f**, **3j**, **4n** and **4o** have shown cytotoxic activity with IC_{50} value falling in the range of <50 μg/mL and compounds **4q** and **4u** have shown cytotoxic activity with IC_{50} value falling in the range of <20 μg/mL. Compounds **3g** and **3h** have shown inhibition in cell proliferation at an IC_{50} value of 4.463 μg/mL and

Table 5
Percentage protection against edema formation

Treatment	Dose (mg/kg)	Percentage protection against edema formation				
		30 min	1 h	2 h	3 h	4 h
Standard (Ibuprofen)	100	38	41.6	52.7	**72.3**	64.4
3g	100	23.5	38.8	50.8	**64.5**	51.6
3h	100	29.4	50.0	67.1	**72.0**	54.9
3i	100	17.6	33.3	47.9	**66.6**	61.5
3j	100	23.5	47.2	64.3	**70.9**	53.8
3k	100	23.5	36.1	54.7	**69.8**	51.6
4n	100	11.7	27.7	42.4	53.7	48.3
4o	100	11.7	30.5	43.8	56.9	50.5
4p	100	11.7	25	39.7	52.6	45
4u	100	23.5	25.0	46.5	**68.8**	47.2
4v	100	23.5	30.5	45.2	**65.5**	56.6

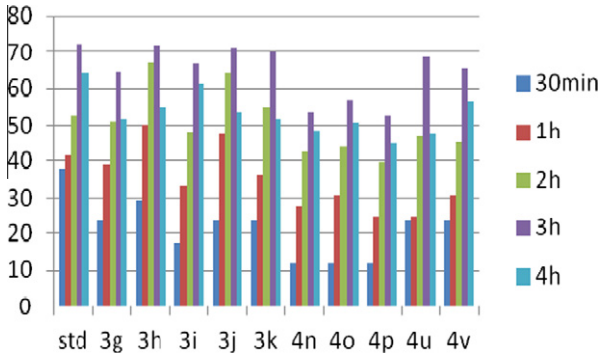


Figure 2. Graphical representation of anti-inflammatory activity.

4.443 μg/mL, respectively whereas **3i** has the most effective compound with IC_{50} = 1.630 μg/mL. Compounds **3g**, **3h** and **3i** are the effective cytotoxic compounds and their percentage inhibitions in cell proliferation against MCF-7 breast cancer cell line were represented graphically as shown in Figure 3.

In summary, an efficient, economical and environmentally benign multicomponent protocol for the construction of pyrano[2,3-*c*]pyrazoles under non-catalytic conditions has been achieved. Pyrano[2,3-*c*]pyrazoles have been evaluated for pharmacological activity profile. Synthesized compounds have shown promising antibacterial and anti-inflammatory activities. In vivo cytotoxic studies are carried out on MCF-7, Human breast tumor cells. Of all the compounds tested, compound **3i** with 3-phenoxyphenyl substitution emerged as the most potent compound. Com-

Table 6
Cytotoxicity of synthesized compounds on MCF-7cell line

S. No.	Test compound	Treatment concentration (μg/mL)	Percentage inhibition in cell proliferation	IC_{50} value[a] (μg/mL)
1	**3e**	1	1.10	45.47
		5	6.96	
		10	16.23	
		25	45.07	
		50	65.64	
2	**3f**	1	0.41	35.414
		5	10.10	
		10	16.33	
		25	25.16	
		50	74.95	
3	**3g**	1	0.28	*4.443*
		2	19.08	
		3	22.03	
		4	41.77	
		5	61.74	
4	**3h**	0.5	5.34	4.463
		1	17.44	
		2	31.88	
		4	42.47	
		6	65.22	
		8	68.64	
5	**3i**	0.1	8.26	1.630
		0.25	13.96	
		0.5	20.25	
		1	43.47	
		2.5	68.64	
		5	83.72	
6	**3j**	1	0.41	42.829
		5	16.33	
		10	34.56	
		25	42.38	

Table 6 (*continued*)

S. No.	Test compound	Treatment concentration (μg/mL)	Percentage inhibition in cell proliferation	IC_{50} value[a] (μg/mL)
		50	50.10	
7	**3k**	1	−1.26	—
		5	−3.16	
		10	0.51	
		25	−5.90	
		50	−4.93	
8	**3m**	1	11.45	—
		5	23.35	
		10	24.37	
		25	24.78	
		50	26.68	
9	**4n**	1	1.67	25.77
		5	22.41	
		10	26.86	
		25	55.49	
		50	84.08	
10	**4o**	1	2.10	47.464
		5	5.96	
		10	22.88	
		25	30.82	
		50	50.23	
11	**4p**	1	0.76	—
		5	4.19	
		10	8.99	
		25	11.57	
		50	29.98	
12	**4q**	1	2.04	15.146
		5	12.51	
		10	30.82	
		25	85.45	
		50	96.42	
13	**4u**	1	19.26	16.766
		5	26.58	
		10	40.26	
		25	64.62	
		50	86.54	

[a] Taxol as a standard reference IC_{50} = 15 nmol.

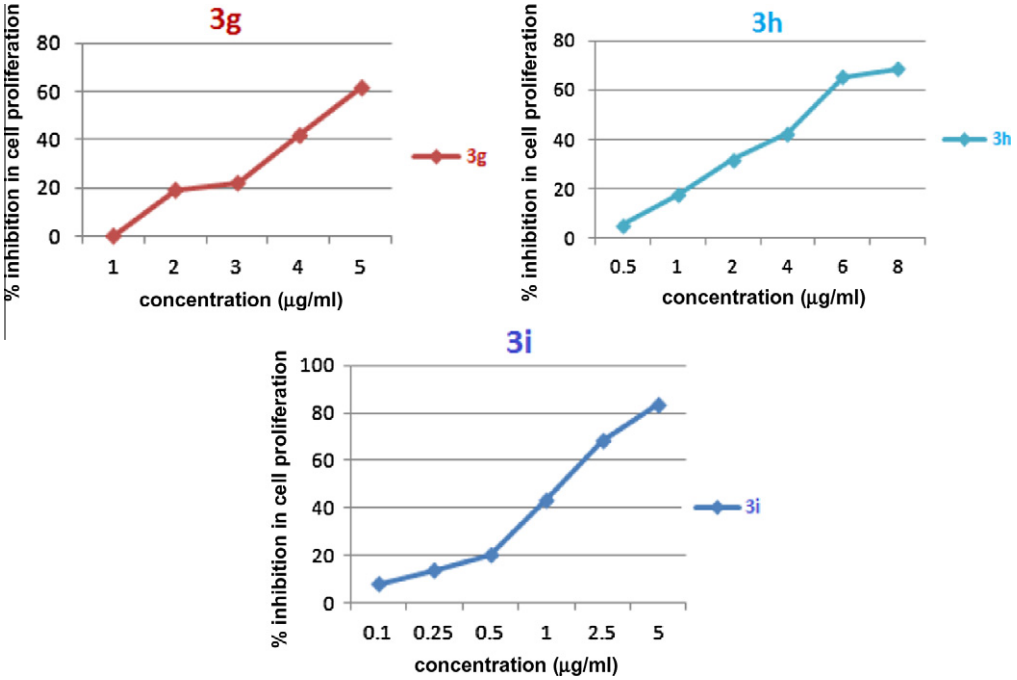


Figure 3. Acute toxicity IC_{50} (μg/mL) of compounds **3g**, **3h** & **3i**.

pound **3i** is a promising lead compound amenable for further improvisation of the activity profile and SAR study.

Acknowledgments

Authors thank Director, I.I.C.T and Head, Crop Protection Chemicals Division, for the facilities and S.R.M. thanks CSIR, New Delhi, for financial support.

Supplementary data

Supplementary data (X-ray crystallographic data, biological evaluation, experimental procedures and spectroscopic data of compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.06.055.

References and notes

1. (a) Smith, W. P.; Sollis, L. S.; Howes, D. P.; Cherry, C. P.; Starkey, D. I.; Cobley, N. K. *J. Med. Chem.* **1998**, *41*, 787; (b) Mazaahir, K.; Shilpi, S.; Khalilur, R. K.; Sharanjit, S. T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4295.
2. Wang, J. L.; Liu, D.; Zheng, Z. J.; Shan, S.; Han, X.; Srinivasula, S. M.; Croce, C. M.; Alnemri, E. S.; Huang, Z. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *97*, 7124.
3. Zaki, M. E. A.; Morsy, E. M.; Abdul, M. *Heterocycl. Commun.* **2004**, *10*, 97.
4. (a) Zaki, M. E. A.; Saliman, H. A.; Hickal, O. A.; Rashad, A. E. *Z. Naturforsch., C: Biosci.* **2006**, *61*, 1; (b) Sheng, C. K.; Li, J. H.; Hideo, N. *J. Med. Chem.* **1984**, *27*, 539.
5. Foloppe, N.; Fisher, L. M.; Howes, R.; Potter, A.; Robertson, A. G. S.; Surgenor, A. E. *Bioorg. Med. Chem.* **2006**, *14*, 4792.
6. (a) Junek, H.; Aigner, H. *Chem. Ber.* **1973**, *106*, 914; (b) Wamhoff, H.; Kroth, E.; Strauch, K. *Synthesis* **1993**, *11*, 1129.
7. Stachulski, A. V.; Berry, N. G.; Low, A. C. L.; Moores, S. L.; Row, E.; Warhurst, D. C.; Adagu, I. S.; Rossignol, J. F. *J. Med. Chem.* **2006**, *49*, 1450.
8. (a) Ramon, D. J.; Yus, M. *Angew. Chem., Int. Ed.* **2005**, *44*, 1602; (b) Zhu, J. *Eur. J. Org. Chem.* **2003**, 1133; (c) Ugi, I.; Domling, A.; Werner, B. *J. Heterocycl. Chem.* **2000**, *37*, 647; (d) Bienayme, H.; Hulme, C.; Oddon, G.; Schmitt, P. *Chem. Eur. J.* **2000**, *6*, 3321; (e) Shinu, V. S.; Sheeja, B.; Purushothaman, E.; Bahulayan, D. *Tetrahedron Lett.* **2009**, *50*, 4838; (f) Arabanian, A.; Mohammadnejad, M.; Balalaie, S.; Gross, J. H. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 887.
9. (a) Chebanov, V. A.; Muravyova, E. A.; Desenko, S. M.; Musatov, V. I.; Knyazeva, I. V.; Shishkina, S. V.; Shishkin, O. V.; Kappe, C. O. *J. Comb. Chem.* **2006**, *8*, 427; (b) Dondoni, A.; Massi, A.; Sabbatini, S.; Bertolasi, V. *J. Org. Chem.* **2002**, *67*, 6979; (c) Rashinkar, G.; Salunkhe, R. *J. Mol. Catal. A: Chem.* **2010**, *316*, 146; (d) Srihari, P.; Singh, V. K.; Bhunia, D. C.; Yadav, J. S. *Tetrahedron Lett.* **2009**, *50*, 3763.
10. Sharanin, Y. A.; Sharanina, L. G.; Puzanova, V. V. *Zh. Org. Khim.* **1983**, *19*, 2609.
11. (a) Mecadon, H.; Rohman, M. R.; Kharbangar, I.; Laloo, B. M.; Kharkongor, I.; Rajbangshi, M.; Myrboh, B. *Tetrahedron Lett.* **2011**, *52*, 3228; (b) Chavan, H. V.; Babar, S. B.; Hoval, R. U.; Bandgar, B. P. *Bull. Chem. Soc.* **2011**, *32*, 3963; (c) Hamad, M. A.; Khaled, D. K.; Aisha, Y. A.; Mohamed, H. E. *Molecules* **2010**, *15*, 6619; (d) Kanagaraj, K.; Pitchumani, K. *Tetrahedron Lett.* **2010**, *51*, 3312; (e) Vasuki, G.; Kumaravel, K. *Tetrahedron Lett.* **2008**, *49*, 5636; (f) Shestopalov, A. M.; Emeliyanova, Y. M.; Shestopalov, A. A.; Rodinovskaya, L. A.; Niazimbetova, Z. I.; Evans, D. H. *Org. Lett.* **2002**, *4*, 423.
12. Nagarajan, A. S.; Reddy, B. S. R. *Synlett* **2002**, 2002.
13. (a) Jaberi, Z. K.; Shams, M. M. R. *Heterocycl. Commun.* **2011**, *17*, 177; (b) Hassan, S.; Maryam, B. *Synth. Commun.* **2009**, *40*, 257; (c) Balaskar, R. S.; Gavade, S. N.; Mane, M. S.; Mane, D. V.; Shingate, B. B.; Shingare, M. S. *Chin. Chem. Lett.* **2010**, *25*, 1175; (d) Heravi, M. M.; Ghods, A.; Derikvand, F.; Bakhtiari, K.; Bamoharram, F. F. *J. Iranian Chem. Soc.* **2010**, *7*, 615.
14. Shailaja, M.; Anitha, M.; Manjula, A.; Rao, B. V. *Indian J. Chem., Sect. B* **2010**, 1088.
15. Mecadon, H.; Rohman, M. R.; Rajbangshi, M.; Myrboh, B. *Tetrahedron Lett.* **2011**, *52*, 2523.
16. Abd-ElLatif, F. F.; Gohar, A. K. M. N.; Fahmy, A. M.; Badr, M. Z. A. *Bull. Chem. Soc.* **1986**, *59*, 1235.
17. Shi, D.; Mou, J.; Zhuang, Q.; Niu, L.; Wu, N.; Wang, X. *Synth. Commun.* **2004**, *34*, 4557.
18. Jin, T.; Wang, A.; Cheng, Z.; Zhang, J.; Li, T. *Synth. Commun.* **2005**, *35*, 137.
19. Latif, F. F. A.; Mekheimer, R.; Ahmed, E. K.; Aleem, T. B. A. *Pharmazie* **1993**, *48*, 736.
20. (a) Deruiter, J.; Carter, D. A.; Arledge, W. S.; Sullivan, P. J. *J. Heterocycl. Chem.* **1987**, *24*, 149; (b) Wang, Z.; Ren, J.; Li, Z. *Synth. Commun.* **2000**, *30*, 763.
21. Tietze, L. F.; Beifuss, U.; Trost, B. M. The Knoevenagel reaction In *Comprehensive Organic Synthesis*; Pergamon Press: Oxford, UK, 1991; Vol. 2, p 341.
22. Bigi, F.; Conforti, M. L.; Maggi, R.; Piccinno, A.; Sartori, G. *Green Chem.* **2000**, *2*, 101.
23. Shestopalov, A. M.; Yakubov, A. P.; Tsyganov, D. V.; Emel'yanova, Yu. M.; Nesterov, V. N. *Chem. Heterocycl. Compd.* **2002**, *38*, 1180.
24. Golubev, A. S.; Pasternak, P. V.; Shidlovskii, A. F.; Saveleva, L. N.; Averkiev, B. B.; Nesterov, V. N.; Antipin, M. Y.; Peregudov, A. S.; Chkanikov, N. D. *J. Fluorine Chem.* **2002**, *114*, 63.
25. *X-ray data of* ***3e***: Formula $C_{14}H_7F_5N_4O$, $M = 342.24$, triclinic, space group $P\bar{1}$, $a = 5.5845(5)$ Å, $b = 7.7381(7)$ Å, $c = 16.2852(15)$ Å, $\alpha = 97.638(1)^\circ$, $\beta = 92.238(2)^\circ$, $\gamma = 94.603(1)^\circ$, $V = 694.36(11)$ Å^3, $Z = 2$, $Dc = 1.637$ g cm^{-3}, μ(Mo K) = 0.154 mm^{-1}, $F(000) = 344$, $T = 294(2)$ K, R1 ($I > 2\sigma(I)$) = 0.0367, wR2 (all data) = 0.1065 for 2171 independent reflections with a goodness-of-fit of 1.036. **CCDC 854873** contains the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0) 1223 336 033; email: deposit@ccdc.cam.ac.uk]. (see Supplementary data).
26. (a) Gunasekaran, P. *Lab. Man. Microbiol.* **1995**, 39; (b) Indian Pharmacopoeia II Ministry of Health and Family Welfare 1996, A-105.
27. NCCLS, Performance Standards for Antimicrobial Susceptibility Testing: Twelfth Information Supplement M, In *National Committee for Clinical Laboratory Standards*, Wayne, PA, 2002; pp 100–512.
28. Parmer, N. S.; Prakash, S. *Screen. Methods Pharmacol.* **2011**.
29. Mosmann, T. *J. Immunol.* **1983**, *65*, 55.