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Diverse synthesis of natural product inspired fused and spiro-heterocyclic scaffolds *via* ring distortion and ring construction strategy

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MCF-7 data of our compounds:

In-vitro cytotoxicity assay was performed with MCF-7 cell line in 96-wells microtitre plates. 30,000 cells/100 µl were seeded per well and allowed to stretch and adhere overnight at 37°C. Following day media was removed and 100 µl fresh media was added to the grown cells. Adhered cells were then treated with scaffolds dissolved in DMSO in triplicates at a standard concentration of 50 µM for 24 hours. We used etoposide at 50 µM as positive control in our experiments. Cytotoxic effect was assessed using ability of live cells to cleave MTT ((3-[4,5- dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide)) (Sigma-Aldrich, St Louis, MO, USA), into formazan crystals. Post 24 hours of treatment 10 µl of MTT (5 mg/ml in PBS) was added to the cells and incubated for 4 hours in dark at 37°C .The violet colored crystals formed were then dissolved in 100 µl DMSO solvent (Dimethyl Sulfoxide, Sigma-Aldrich, St Louis, MO, USA). The colorimetric assay was read at 570nM wavelength in spectrophotometer. Percentage inhibition was calculated using the following formula:

$$\% \text{ inhibition} = (\text{Avg O.D}_{\text{control}} - \text{Avg O.D}_{\text{treatment}} / \text{Avg O.D}_{\text{control}}) * 100$$

Compounds	Reading1	Reading 2	Reading 3	Std. Dev.	avg O.D	ctrl-test	ctrl-test/ctrl	*100
control	1.8690	1.8270	1.8810	0.0284	1.8590	0.0000		
etoposide	1.0500	1.0700	1.1080	0.0294	1.0760	0.7830	0.4212	42.1194
5	1.7200	1.7600	1.9900	0.1457	1.8233	0.0357	0.0192	1.9186
10	1.3480	1.3740	1.3150	0.0296	1.3457	0.5133	0.2761	27.6134
13	1.7840	1.6070	1.8360	0.1201	1.7423	0.1167	0.1084	10.8426
11	1.8480	1.6210	1.8390	0.1285	1.7693	0.0897	0.0492	4.9177
9	2.0130	1.9480	1.7970	0.1108	1.9193	-0.0603	-0.0448	-4.4835
12	2.2950	1.5340	1.9130	0.3805	1.9140	-0.0550	-0.0316	-3.1567
7	2.5090	2.0010	1.9220	0.3186	2.1440	-0.2850	-0.1611	-16.1078
8	2.4250	1.6660	2.0500	0.3795	2.0470	-0.1880	-0.0980	-9.7951
14	1.9440	1.9640	2.1430	0.1096	2.0170	-0.1580	-0.0825	-8.2550

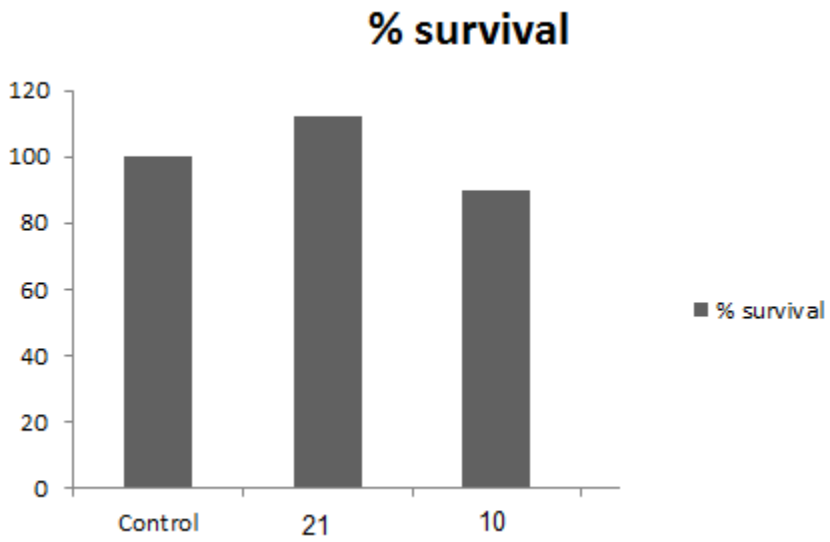
6	1.6820	1.4940	1.6010	0.0943	1.5923	0.2667	0.1244	12.4378
15a	1.9560	1.9860	1.8470	0.0731	1.9297	-0.0707	-0.0345	-3.4522
15b	2.1880	1.7870	1.8960	0.2073	1.9570	-0.0980	-0.0486	-4.8587
16	1.8203	1.7141	1.6466	0.0875	1.7233	0.1357	0.0716	7.3158
21	1.2532	1.3076	1.1687	0.0699	1.2367	0.6223	0.3346	33.4589
22	1.5981	1.6720	1.6301	0.0371	1.6347	0.2243	0.1207	12.0674
23	2.2411	2.3132	2.3641	0.0618	2.3033	-0.4443	-0.2390	-23.9017

48h screening against MCF-7 of **10 and 21**

		MCF7 (1st assay)					MCF7 (2nd Assay)				
		Reading 1	Reading 2	avg O.D	ctrl-test	% inhibition	Reading 1	Reading 2	avg O.D	ctrl-test	% inhibition
control		1.291	1.312	1.3015	0		0.952	0.988	0.97	0	
Etoposide		0.652	0.591	0.6215	0.68	52.24740684	0.516	0.501	0.5085	0.4615	47.57731959
21		0.818	0.789	0.8035	0.498	38.26354207	0.515	0.536	0.5255	0.4445	45.82474227
10		0.764	0.751	0.7575	0.544	41.79792547	0.602	0.625	0.6135	0.3565	36.75257732

COS-7 data for 10 and 21:

Methodology: To evaluate cytotoxicity of the effective scaffolds on non-carcinoma cell line we performed MTT assay with COS-7 cells at the same concentration used for screening i.e. 50 µM. Briefly, 30,000 cells per well were seeded in a microtitre plate and treated with compounds for 24 hour time interval. Cytotoxicity was measured in terms of reduction of MTT to purple color formazan crystals.



Principal Component Analysis

A set of 1555 FDA-approved drugs in smiles format was download from Drug Bank [1] and 746 chembridge screen compound were obained from the literature [2]. Also the informationn of FDA-approved drugs for breast cancer were retrieved from National Cancer Institute. Diversity of our library compounds were studied using various shape based and charge based molecular surface descriptors i.e TAE/RECON electrostatic potential and electrostatic potential autocorrelation descriptors [2] as described in Albert Isidro-Llobeta [3]. Further Principal Component analysis is done that helps in determing the overall chemical space. Principal component analysis (PCA) is a multi-variate statistical method that reduces dimensionality of a data while retaining most variation present in the data. This is done by transforming the original data to a new set of uncorrelated variables known as principal components (PC). Few of these principal components can be used to represent a molecule rather than by thousands of variables. PCA analysis is widely used in chem-informatics to visualize the multi-dimensional chemical space. This multi-dimensional chemical space is defined by the molecular descriptors calculated for every molecule. PCA reduces the higher dimensional chemical space to a lower dimensional chemical space which is then easy to visualize and helps in extracting various drug-likeness information from this space. All the descriptors calculation and PCA analysis was done in Molecular Operating enviornment software.[4]

References

1. Wishart DS, et al. (2008) DrugBank: A knowledgebase for drugs, drug actions and drug targets. Nucleic Acids Res 36:D901–D906.
2. Breneman CM, et al. (2003) New developments in PEST shape/property hybrid descriptors. J Comput Aid Mol Des 17:231–240.
3. Isidro-Llobeta, et al. (2011) Diversity-oriented synthesis of macrocyclic peptidomimetics. PNAS vol. 108, no. 17, 6793- 6798.
4. Molecular Operating Environment (MOE), 2013.08; Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2015.

Single crystal X-Ray data collection

Single crystal X-ray diffraction data on compound **11** and **12** were collected at room temperature using Bruker AXS Apex II diffractometer systems equipped with a CCD detector. The measurement method employed in this cases was omega and phi scan. Whereas for compound **15a** the data was collected at 150 K using XtaLABmini: Fixed Chi 2 circle diffractometer system equipped with CCD detector. In this case the measurement method adopted was omega scan only. In all the three cases the X-ray source used was Mo K_α (λ = 0.71073 A) and the crystal to detector distance was maintained at 50 mm. The crystal structures were solved by means of direct methods and refined with full-matrix least squares on SHELXL-2014. All the hydrogen atoms were placed at calculated positions and refined using a riding model with appropriate HFIX commands. The structural details can be found from the deposited CIF with CCDC numbers 1014865, 1056222 and 1056225 for **11**, **12** and **15a** compounds, respectively.