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COMMUNICATION

Diverted organic synthesis (DOS): accessing a new, natural product inspired, neurotrophically active scaffold through an intramolecular Pauson–Khand reaction†

Goverdhan Mehta,*[a] Ramesh Samineni,[b] Pabbaraja Srihari,*[b] R. Gajendra Reddy[c] and Sumana Chakravarty[c]

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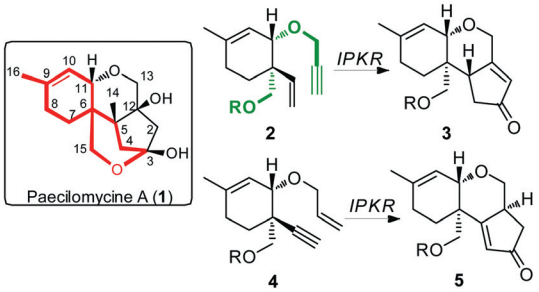
Drawing inspiration from the impressive neurotrophic activity exhibited by the natural product paecilomycine A, we have designed a new natural product-like scaffold employing an intramolecular Pauson–Khand reaction. Several compounds based on the new designer scaffold exhibited promising neurotrophic activity and are worthy of further biological evaluation. Our findings also highlight the importance of a DOS strategy in creating useful therapeutical leads.

Diverted organic synthesis (DOS) and diverted total synthesis (DTS) are powerful strategies for creating chemical diversity and for navigation through unexplored chemical space.[1] DOS and DTS are not mutually exclusive terms and encompass an arena that enables access to complex, non-obvious, high-pedigree scaffolds for therapeutic profiling. While DOS is an opportunistic tactic that may derive inspiration from natural product synthesis efforts to generate new molecular ensembles, DTS is essentially a modality to access edited and reformed variants of a promising natural product lead structure, through an advanced intermediate in its synthesis, without compromising the integrity of natural product core architecture. However, in essence, both DOS and DTS are adaptive and productive off-shoots of a total synthesis endeavour.

One of the emerging healthcare challenges posed by the aging world population is the maintenance of cognitive functions and the ability to treat neurodegenerative disorders like dementia and Alzheimer's.[2] In this quest, chemical entities that can repair and regenerate neurons hold the key. Nature does provide such a mechanism in the form of peptide based neurotrophins like NGF, BDNF, NTF3, *etc*. but their action and role needs to be sustained, stimulated and augmented. In recent years, several small molecule natural products (SMNPs) have been found to promote neurite growth and synaptic plasticity by up-regulating the activity of neurotrophins.[3] These observations, besides providing useful leads for developing therapeutic agents for slowing down neurodegeneration and treating related disorders, underscore the pressing need for synthetic chemists' intervention through DOS and DTS to design new scaffolds based on natural product leads and to amplify natural product diversity.[1,3]

For some time now, our group[4] has been actively engaged in the synthesis of complex, diverse and neurotrophically active natural products. As part of this endeavour, we[5] and others[6] have recently reported a synthesis of paecilomycine A **1**, a tricothecane derived sesquiterpenoid natural product, shown to enhance and promote impressive (10 nM concentration) neurite outgrowth in PC-12 cells.[7] In our synthesis of paecilomycine A and analogues, an intramolecular Pauson–Khand reaction (IPKR) between tethered vicinal alkyne and alkene side arms was the pivotal step[5] (**2** → **3** and **4** → **5**) to generate its core architecture, Scheme 1.

Impressed by the exceptional neurotrophic activity exhibited by **1**, we felt encouraged to initially generate[5a] diversity around its core structure and then to extend the key IPKR strategy to create a new bioactive scaffold. In this context, we recognized the presence of a 2-oxa-spiro[5.5]undecane segment (see



Scheme 1 IPKR based strategy for the synthesis of **1**.

[a]*School of Chemistry, University of Hyderabad, Hyderabad-500 046, India. E-mail: gmsc@uohyd.ernet.in, gm@orgchem.iisc.ernet.in; Fax: +91 4023012460; Tel: +91 4023010785*
[b]*Division of Natural Products Chemistry, CSIR-Indian Institute of Chemical Technology, Hyderabad-500 007, India*
[c]*Chemical Biology/Division of Natural Products Chemistry, CSIR-Indian Institute of Chemical Technology, Hyderabad-500 007, India*

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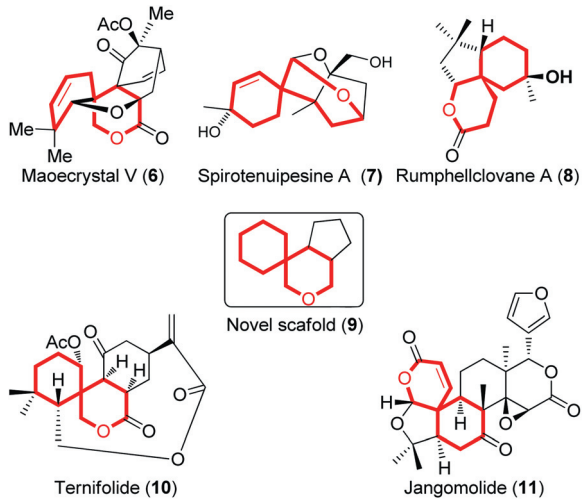
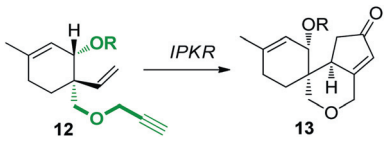


Fig. 1 Few natural products that contain spiro-scaffold **9**.

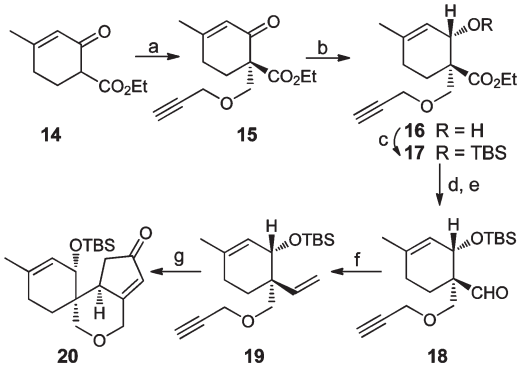
highlighted portion) embedded within the paecilomycine A **1** framework. Notably, a 2-oxa-spiro[5.5]undecane sub-structure is also present in many important natural products of contemporary therapeutic interest like maoecrystal V **6**,[8] spirotenuipesine A **7**,[9] rumphellclovane **8**,[10] ternifolide **10**[11] and jangomolide **11**[12] as part of their interlaced molecular architecture (Fig. 1). Taking a cue from this observation, we decided to design a new scaffold that incorporates a 2-oxa-spiro[5.5]undecane fragment in a 'paecilomycine A **1**' like structural ambiance. We describe here a short, straightforward access to a 2-oxa-spiro[5.5]undecane based conceptualization **9** and observe that several derivatives based on this scaffold exhibit impressive neurotrophic activity.

Generation of the new scaffold **9** was envisaged through an adaptation of the key IPKR steps (**2** → **3** and **4** → **5**) in our synthesis of the paecilomycine A. It was envisioned that by employing a variant **12** of **2**, with tethered enyne moieties occupying geminal positions, on IPKR should deliver 'paecilomycine A-like' **13** that embodies the 2-oxa-spiro[5.5]undecane fragment, Scheme 2. From synthetic strategy considerations, precursor **12** was derivable by simply 'swapping' the propargyl and OTBS groups in **2**. Thus, accessing **12** was our first objective and a route to it was devised by creating an early diversion within our synthesis of the natural product **1**.

Ethyl 4-methyl-2-oxocyclohex-3-enecarboxylate **14**, previously deployed[5a] in our earlier foray towards paecilomycine A synthesis, was subjected to propargyloxy-methylation with (propargyloxy)methyl chloride[13] to furnish **15**, Scheme 3. Luche reduction[14] in **15** was stereoselective and furnished α-hydroxy compound **16** in which the hydroxyl group was protected as TBS ether **17**. Ester reduction in **17** with DIBAL-H and IBX oxidation furnished the aldehyde **18**. Wittig olefination in **18** was uneventful and furnished the required IPKR precursor enyne **19**. The stage was now set for the key intramolecular Pauson–Khand reaction and exposure of **19** to $Co_2(CO)_8$ furnished the spiro-fused tricyclic enone **20** as a single diastereomer in a decent 75% yield, Scheme 3. Stereostructure of **20** was secured through single crystal‡ X-ray structure determination and it was interesting to note that intramolecular [2 + 2 + 1]-cycloaddition (IPKR) occurred preferentially from the β-face. An ORTEP diagram of



Scheme 2 Key step for generating the new spiro-fused scaffold.



Scheme 3 Synthesis of tricyclic spiro-fused scaffold **20**. *Reagents and conditions*: (a) NaH, HMPA, THF, 3-(chloromethoxy)prop-1-yne, TBAI, −10 °C, 3 h, 74%; (b) $CeCl_3{\cdot}7H_2O$, $NaBH_4$, MeOH, −15 °C, 15 min, 82%; (c) TBSCl, imidazole, CH_2Cl_2, 8 h, 84%; (d) DIBAL-H, 0 °C, 1 h, CH_2Cl_2, 93%; (e) IBX, DMSO, THF, rt, 2 h, 90%; (f) ^{t}BuOK, PPh_3CH_2I, THF, 0 °C, 15 min, 69%; (g) (i) $Co_2(CO)_8$, CH_2Cl_2, 1 h; (ii) NMO, CH_2Cl_2, 4 h, 75%.

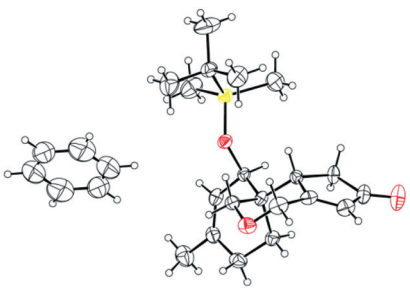
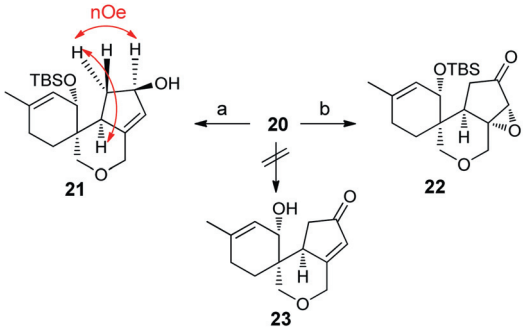
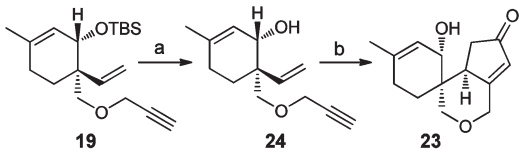


Fig. 2 ORTEP diagram of **20**.

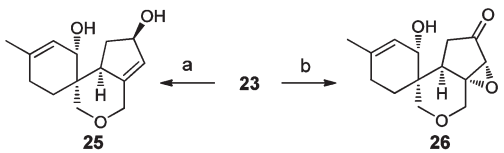
20 is displayed in Fig. 2. To generate variegated functionalities on the core structure, we carried out Luche reduction[14] in **20** to deliver stereoselectively the allylic alcohol **21** (stereochemistry at the newly generated centre was secured from NOESY analysis). An epoxide **22** could be readily prepared from **20** *via* nucleophilic epoxidation,[15] Scheme 4. However, TBS deprotection in **20** to deliver hydroxyl-enone **23** proved to be unexpectedly problematic and an alternative way was sought. It was observed that TBS deprotection in the IPKR precursor enyne **19** was no issue at all and the resulting **24** could be induced to undergo a stereoselective intramolecular Pauson–Khand reaction to furnish the spiro-fused tricyclic hydroxy-enone **23**, Scheme 5. Two derivatives of **23** were prepared stereoselectively through Luche reduction to tricyclic diol **25** and nucleophilic epoxidation to epoxide **26** for bioassay purposes, Scheme 6.



Scheme 4 Functional group modifications on **20**. *Reagents and conditions*: (a) $CeCl_3{\cdot}7H_2O$, $NaBH_4$, MeOH, −15 °C, 15 min, 78%; (b) H_2O_2, 6 N NaOH, MeOH, 0 °C, 2 h, 83%.



Scheme 5 Synthesis of hydroxy-enone **23**. *Reagents and conditions*: (a) TBAF, THF, rt, 12 h, 92%; (b) (i) $Co_2(CO)_8$, CH_2Cl_2, 1 h; (ii) NMO, CH_2Cl_2, 6 h, 79%.



Scheme 6 Functional group modifications on **23**. *Reagents and conditions*: (a) $CeCl_3{\cdot}7H_2O$, $NaBH_4$, MeOH, −15 °C, 15 min, 74%; (b) H_2O_2, 6 N NaOH, MeOH, 0 °C, 2 h, 78%.

Biological evaluation

With six new compounds **20**, **21**, **22**, **23**, **25** and **26**, of secured stereostructures and based on the novel scaffold **9** in hand, attention was turned towards profiling their CNS activity using Neuro2a cell lines.[16,17] Interestingly, all the six compounds **20–23**, **25** and **26**, embodying the 2-oxa-spiro[5.5]undecane fragment, were found to be neuroprotective in standard MTT assay and Trypan blue assay for cell viability.[16,18,19] Encouraged by this preliminary observation, we further examined the ability of our compounds to stimulate neurite outgrowth in Neuro2a cells following the standard cell culture method.[16,18] Cells were grown accordingly and then exposed to the compounds (**20**, **21**, **22**, **23**, **25** and **26**) along with NGF (positive control) and natural product honokiol (a standard reference for neurotrophic activity) at different concentrations for 48 h. All compounds were diluted in DMSO and its concentration in the culture medium was not more than 0.1%, a concentration threshold at which DMSO did not affect cell growth or death. Cells were incubated for ~48 h and analyzed for neurite outgrowth under a microscope using ImageJ software.

Table 1 Morphometric analysis of neurite outgrowth of differentiated Neuro2a cells cultured for 48 h in DMSO (0.1%), compounds (0.01 μM). The tabulated data and bar graphs were expressed as mean ± SEM where $n = 60$ and $*p < 0.01$ *versus* control

Treatment	Average neurite length per neuron (μM)	Percent change from control
Control	39.70 ± 2.41	—
DMSO	42.54 ± 2.35	7.15
NGF(100 ng mL^{-1})	63.68 ± 2.73	60.40
Honokiol (1 μM)	59.59 ± 3.35	50.10
20 (0.01 μM)	53.56 ± 3.10	34.91
21 (0.01 μM)	47.36 ± 3.20	19.29
22 (0.01 μM)	45.07 ± 2.85	13.53
23 (0.01 μM)	58.64 ± 4.24	47.70
25 (0.01 μM)	61.78 ± 2.89	55.62
26 (0.01 μM)	56.19 ± 3.62	41.54

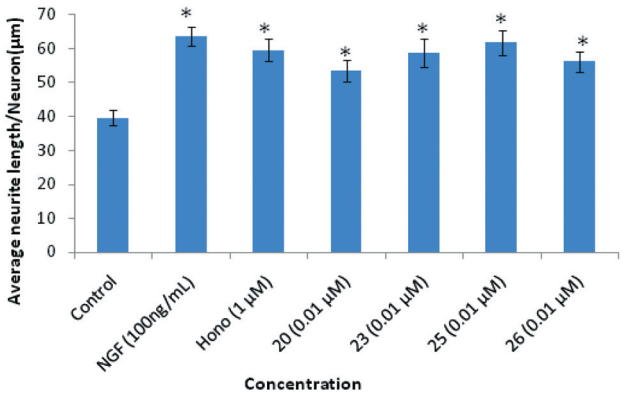


Fig. 3 Bar representation of the neurite outgrowth of differentiated Neuro2a cells by the more active compounds **20**, **23**, **25**, **26** and comparison with control and NGF and honokiol.

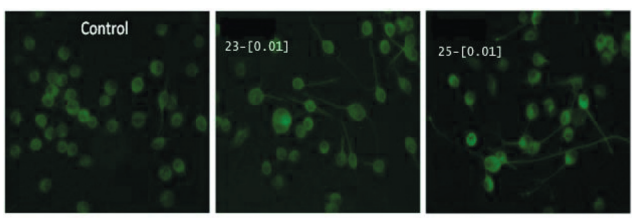


Fig. 4 Immunocytochemical images stained with beta III tubulin showing optimum changes in neurite outgrowth of differentiated Neuro2a cells with control, **23** and **25**, respectively.

Table 1 and Fig. 3 display an increase in neurite length per neuron (average of 60 neurons for each compound) after 48 h of exposure to the test compounds along with the controls. Gratifyingly, the percentage change in neurite outgrowth mediated by our compounds was quite comparable to that induced by NGF and honokiol, Table 1. However, it was quite remarkable to find that compounds based on our designer scaffold exhibited activity comparable with honokiol at ~100-times lower concentration, Fig. 3. Significant neurite outgrowth affected by representative designer compounds **23** and **25** on Neuro2a cells *vis-à-vis* the control can be seen in Fig. 4.

Conclusions

Our quest for novel structures that can exhibit neurotrophic activity, inspired by the natural product paecilomycine A, has led to the design of a new scaffold based on the 2-oxa-spiro[5.5]-undecane framework. The newly conceptualized scaffold and many of its derivatives have been accessed through a concise IPKR based strategy and attendant functional group modifications. It was gratifying to note that all the compounds based on the 2-oxa-spiro[5.5]undecane scaffold were neuro-protective and exhibited noteworthy neurotrophic activity, warranting further developmental efforts in the area.

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Notes and references

‡ Single crystal X-ray diffraction data were collected on a Bruker AXS SMART APEX CCD diffractometer at 291 K using graphite monochromated $MoK_{\alpha 1}$ radiation (λ = 0.7107 Å). The data were reduced by *SAINTPLUS*; an empirical absorption correction was applied using the package *SADABS* and *XPREP* was used to determine the space group. The crystal structures were solved by direct methods using *SIR92* and refined by a full-matrix least-squares method on F^2 using *SHELXL97*. Crystal data for **20**: $C_{20}H_{32}O_3Si\cdot\frac{1}{2}(C_6H_6)$, M = 387.6, triclinic, $P\bar{1}$, a = 7.5526(11), b = 8.7285(13), c = 17.680(3) Å, α = 85.539(3), β = 82.149 (3), γ = 87.533(3)°, V = 1150.4(3) Å^3, Z = 2, ρ_{calcd} = 1.119 g cm^{-3}, 8616 reflections measured, 4204 unique (R_{int} = 0.0718), R_1 = 0.1084 and wR_2 = 0.2088 for 2233 observed reflections, CCDC-876473.

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