Organic & Biomolecular Chemistry

COMMUNICATION

View Article Online
View Journal

Cite this: DOI: 10.1039/c6ob01351a

Received 22nd June 2016,
Accepted 1st August 2016

DOI: 10.1039/c6ob01351a

www.rsc.org/obc

Synthesis of "neoprofen", a rigidified analogue of ibuprofen, exemplifying synthetic methodology for altering the 3-D topology of pharmaceutical substances†

Ron R. Ramsubhag,[a] Chelsea L. Massaro,[a] Christina M. Dadich,[a] Andrew J. Janeczek,[a] Tung T. Hoang,[a] Elizabeth A. Mazzio,[b] Suresh Eyunni,[b] Karam F. A. Soliman[b] and Gregory B. Dudley*[a,c]

3,3-Dimethylcyclopentanes (neopentylenes) are ubiquitous in Nature but largely absent from synthetic pharmaceutical libraries. Neopentylenes define a hydrophobic and rigid 3-D topology with distinct molecular pharmacology, as exemplified here with two neopentylene-fused analogues of the synthetic anti-inflammatory drug, ibuprofen.

Rigid structural features lend themselves to specific biomolecular interactions. This Communication features preliminary results from synthetic efforts focused on the installation of the neopentylene ring fusion (*i.e.*, in polycyclic 3,3-dimethyl-cyclopentanes), a rigid and hydrophobic structural motif that, in principle, can enhance the three-dimensional topology of known and novel pharmacophores.

Neopentylene ring-fused compounds are ubiquitous in Nature (*cf.* Fig. 1, top), but synthetic variants are largely absent from pharmaceutical screening libraries and drug discovery programs. Illudol[1] and pentalenene,[2] for example, have long stood as challenging targets for total synthesis, in part due to the difficulty of crafting the hydrophobic neopentylene ring fusion. Syntheses of tremulenolide A,[3] illudalic acid,[4] and alcyopterosin A[5] pose similar problems. Medicinal chemistry efforts associated with illudalic acid and alcyopterosin A focused on simplified synthetic analogues in which the neopentylene ring fusion was deleted[6] or truncated,[7] respectively, for synthetic convenience. The structurally simplified analogues were universally less potent than their neopentylene-fused congeners.

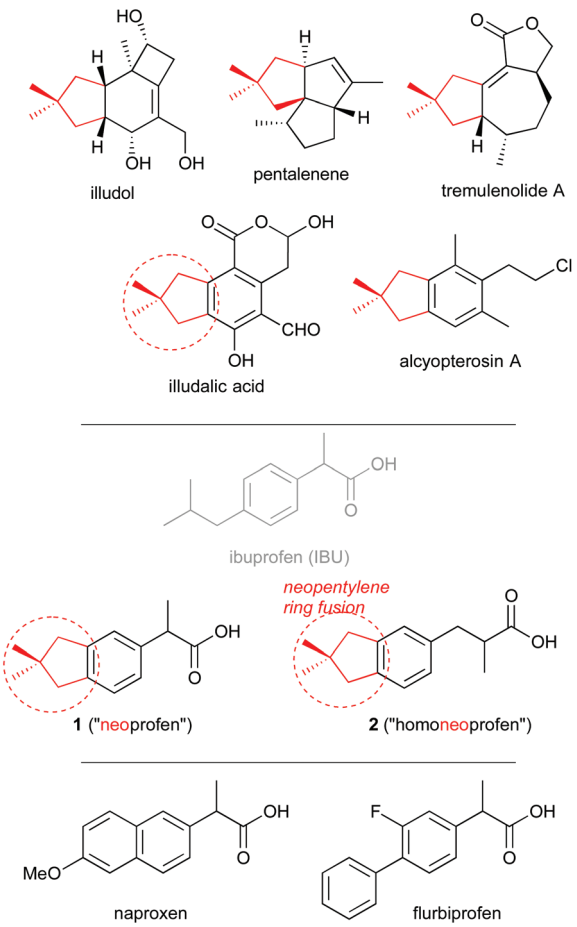


Fig. 1 Top: Natural products featuring a neopentylene ring fusion. Middle: Ibuprofen (IBU) and "neoprofen" (**1**), the current focus. Bottom: Naproxen and flurbiprofen.

One rare example of a synthetic neopentylene is the ibuprofen analog **1** (Fig. 1, middle), in which the 4-isobutyl substituent has been replaced with a 3,4-neopentylene ring.

[a]*Department of Chemistry and Biochemistry, Florida State University, Tallahassee, Florida 32306-4390, USA. E-mail: gdudley@chem.fsu.edu*
[b]*College of Pharmacy and Pharmaceutical Sciences, Florida Agricultural and Mechanical University, Tallahassee, Florida 32307, USA*
[c]*C. Eugene Bennett Department of Chemistry, West Virginia University, Morgantown, West Virginia 26506, USA*

†Electronic supplementary information (ESI) available: Experimental procedures, characterization and molecular docking data, copies of NMR spectra. See DOI: 10.1039/c6ob01351a

Ibuprofen analog **1**, which we refer to herein as "neoprofen", has been prepared in 9 steps from benzaldehyde as described in a 1979 patent.[8] The pharmacology of synthetic neopentylenes is largely unexplored; our central hypothesis is that the neopentylene provides a rigid, topologically unique, and hydrophobic anchor that perturbs biomolecular interactions relative to truncated and/or more conformationally flexible analogues. Here we offer some initial validation of this central hypothesis in the context of neoprofen (**1**) and "homo-neoprofen" (**2**), including preliminary pharmacological activity reminiscent of but not identical to ibuprofen (IBU).

The preliminary pharmacological assessments described herein are understood in the context of known structure–activity relationship (SAR) observations regarding the IBU core. For example, naproxen[9] and flurbiprofen[10] (Fig. 1, bottom) are both better inhibitors of the COX-2 enzyme than is ibuprofen. Based on X-ray crystal data, molecular docking, and mutagenesis studies, the latter substances (naproxen and flurbiprofen) bind analogously to IBU in the COX-2 binding site, with the differences in affinity associated with variable extension of hydrophobic aromatic regions of the molecules into a hydrophobic enzyme pocket.[11] Neoprofen is more massive but compact and conformationally restricted relative to IBU, so it is not expected to extend as deeply into the hydrophobic pocket of the COX-2 enzyme.[12]

Our synthesis of neoprofen (**1**, Scheme 1) draws on tandem fragmentation/olefination methodology[13] for the synthesis of neopentyl-tethered 1,6-enynes, which are suitable for preparing diverse synthetic neopentylenes by strategic application of myriad enyne cycloisomerization and annulation methods. Here we expand this methodology to tandem fragmentation/*vinylogous* olefination to prepare neopentyl-tethered dienyne **5** in three steps from commercially available dimedone. Addition of methyllithium to **5** is followed by a rhodium-catalyzed intramolecular [4 + 2] formal Diels–Alder reaction,[14] with subsequent DDQ oxidation to give rise to indane **7**. We employed a similar sequence of Rh-catalyzed cycloisomerization and oxidation in our recent synthesis of alcyopterosin A.[15] Here, tertiary alcohol **7** was formally rearranged to primary alcohol **9** by dehydration followed by hydroboration/oxidation. Finally, two-stage oxidation provided neoprofen (**1**).

Our synthesis of neoprofen was guided by the hypothesis that the neopentylene ring would perturb pharmacologically relevant interactions relative to ibuprofen. Computational modelling provided insight into the potential validity of this hypothesis and guided the design of a second ibuprofen analog. Computationally overlaying ibuprofen (IBU) and **1** in the cyclooxygenase II (COX-2) enzyme binding pocket illustrated how the compounds may bind (Fig. 2). The carboxylic acid of IBU forms a salt bridge with arginine 121 of the COX-2 enzyme, locking the molecule in place. The isobutyl tail of IBU extends into a hydrophobic pocket formed by phenylalanine and tyrosine residues. Analog **1** can form a similar salt bridge with arginine 121. However, the neopentylene ring of **1** is more rigid and compact than the isobutyl group of IBU, such that the neopentylene ring cannot extend as deeply into the hydrophobic pocket as the isobutyl group.

As noted above, our central hypothesis is that the neopentylene ring of **1** will perturb pharmacological interactions relative to the more conformationally flexible (and more linear) isobutyl group of IBU. We therefore expected there to be observable differences in the molecular pharmacology of IBU and **1**. As a logical extension of these hypotheses, we

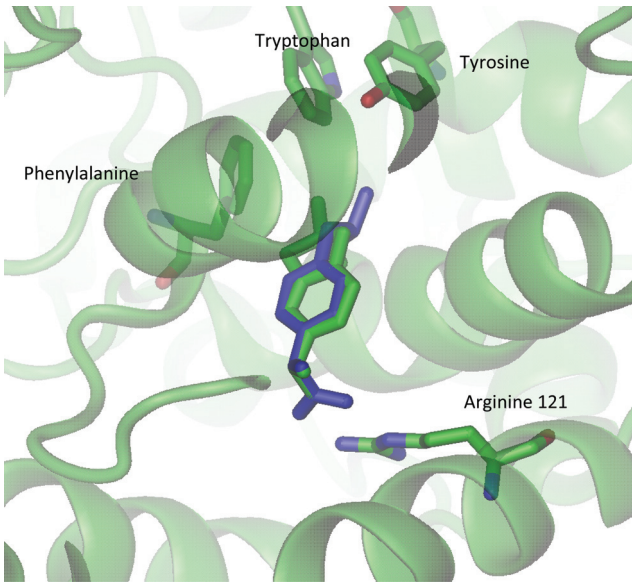
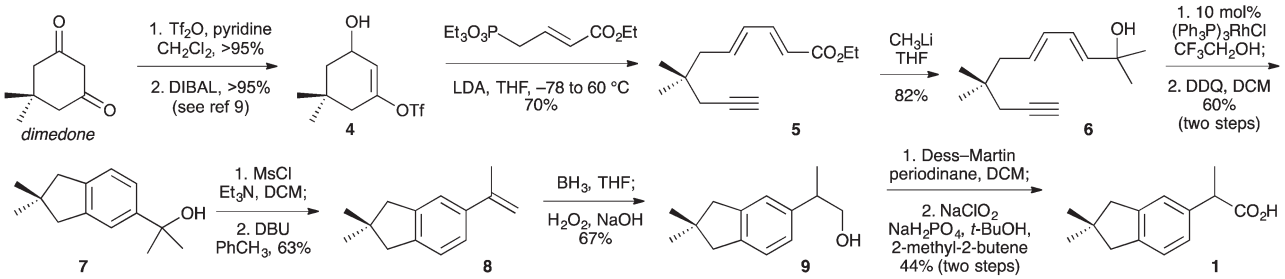


Fig. 2 Ibuprofen (IBU, blue) *vs.* neoprofen (**1**, green) overlaid in the COX-2 enzyme pocket.



Scheme 1 Synthesis of neoprofen (**1**) (see ESI† for details).

reasoned that if we could lengthen and restore some conformational flexibility to **1**, then we might observe less of a difference in molecular pharmacology as compared to IBU. Therefore, we designed and synthesized "homoneoprofen" **2** (*cf.* Fig. 1), which we reasoned would penetrate deeper into the hydrophobic pocket (by analogy to naproxen or flurbiprofen) than **1** and may offset perturbations in binding associated with the switch from the isobutyl chain of IBU to the neopentylene ring of **1**. Additional molecular docking studies on murine cyclooxygenase II (COX-2) suggest that analogues **1** and **2** should bind COX-2 in the same pocket as IBU (see ESI† for details).

To obtain "homoneoprofen" (**2**, Scheme 2), we started from dienyne **5** (*cf.* Scheme 1). DIBAL-H reduction gave primary allylic alcohol **10**. Oxidative cycloisomerization was again accomplished in a two-stage process using Wilkinson's catalyst in TFE, followed by addition of DDQ. In this case, however, DDQ treatment resulted in a productive secondary oxidation of the expected primary alcohol to aldehyde **10a**. Aldehyde **10a** was isolated but not purified before being converted to unsaturated ester **11** using Horner–Wadsworth–Emmons conditions. Hydrogenation of **11** gave saturated ester **12**, and finally saponification provided homoneoprofen **2**.

With **1** and **2** in hand, we proceeded to sample their activity in the human COX-2 enzyme and relative to IBU (Fig. 3). Compared to ibuprofen (IC_{50} 0.02 μg mL^{-1} or 1 μM in our assays), neoprofen **1** showed relatively poor activity: IC_{50} 4 μg mL^{-1} (20 μM). This observation is consistent with our central hypothesis: replacing the isobutyl side chain with a neopentylene ring perturbs molecular pharmacological interactions. In this case, activity was reduced as a consequence of the rigid, compact neopentylene ring. The intermediate activity of homoneoprofen **2** (IC_{50} 0.4 μg mL^{-1} or 2 μM; *i.e.*, between that of IBU and **1**) is consistent with our design criteria for **2** of restoring two-dimensional length and conformational flexibility so as to extend more deeply into the COX-2 binding pocket than does neoprofen (**1**). These observations are qualitatively consistent with the SAR trends associated with naproxen[9,11] and flurbiprofen.[10]

In conclusion, we have designed, prepared, and analysed two neopentylene-fused analogues of ibuprofen, toward the long-term goal of understanding the unique hydrophobic 3-D topology of the neopentylene ring fusion and its potential role in drug discovery. Neopentylene ring-fused polycyclic structures are ubiquitous in Nature but largely absent from modern synthetic pharmaceutical screening libraries. The omission of neopentylene-fused molecular substances is perhaps related to historical limitations in the synthetic chemistry of neopentyl-tethered bifunctional building blocks; current and on-going methodology in these laboratories is aimed at addressing and overcoming these limitations. The synthesis of so-called neoprofen (**1**) and homoneoprofen (**2**) reflect the difficulties associated with preparing neopentylene-fused compounds—more work is certainly needed in this area—but these compounds also highlight the potential pharmacological significance of this compact, rigid, hydrophobic, and topologically unique structural feature. Although neoprofen (**1**) is higher molecular weight than ibuprofen by virtue of its one extra carbon atom, neoprofen (**1**) seemingly cannot occupy as much conformational space as ibuprofen in the relevant COX-2 binding pocket due. Preliminary support for this tentative interpretation of the assay data comes from the broader SAR associated with naproxen and flurbiprofen as well as the restored binding affinity of homoneoprofen (**2**), which adds to the deviation from ibuprofen in terms of molecular weight but restores the 2-D length and conformational flexibility that was found to be advantageous in molecular docking simulations (see ESI† for details). On the other hand, the rigidity and compact nature of **1** (and other neopentylenes) should be generally advantageous for ligation in compact and rigidly defined binding pockets. Therefore, we anticipate that neopentylene

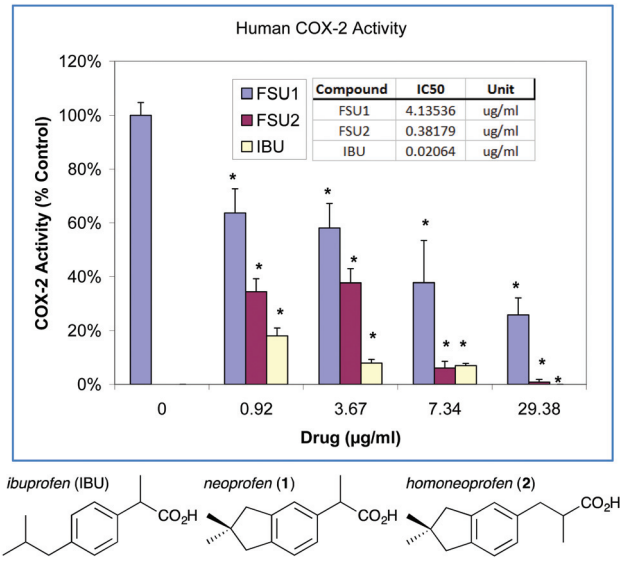
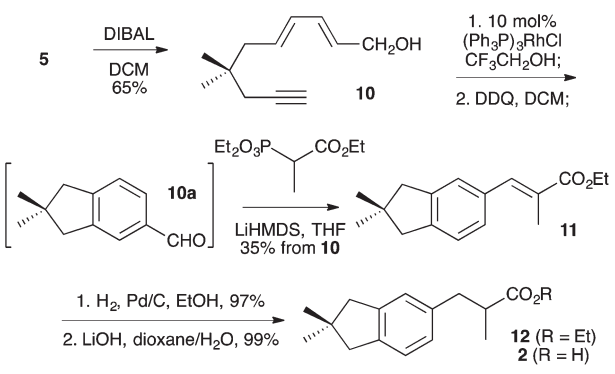


Fig. 3 Human COX-2 activity inhibition assay; activity of homoneoprofen (**2**, FSU2, red bar) was intermediate between that of ibuprofen (IBU, yellow bar) and neoprofen (**1**, FSU1, blue bar). The data represent COX-2 activity (% control) and are presented as the mean ± st. dev, n = 2. Statistical difference from the controls were determined by a students t-test. *P < 0.05 (see ESI† for details).



Scheme 2 Synthesis of homoneoprofen (**2**) (see ESI† for details).

ring-fused structures should have strategic value in molecular pharmacology.

This research was supported by grants from the National Science Foundation (NSF-CHE 1300722) and the National Institute of Minority Health and Health Disparities of the National Institutes of Health through Grant Number 8 G12MD007582-28 and Grant Number 1P20 MD006738-01. We thank Prof. Wei Yang (FSU) for helpful discussions and assistance with molecular modelling.

Notes and references

1 T. C. McMorris, M. S. R. Nair and M. Anchel, *J. Am. Chem. Soc.*, 1967, **89**, 4562–4563.
2 B. K. Koe and A. B. Sobin, *Antibiot. Annu.*, 1957, 672.
3 W. A. Ayer and E. R. Cruz, *J. Org. Chem.*, 1993, **58**, 7529.
4 J. F. Ammirati, *Poisonous Mushrooms of the Northern United States and Canada*, E. P. Dutton, New York, N.Y., 1972, p. 84.
5 J. A. Palermo, M. F. Rodriguez Brasco, C. Spagnuolo and A. M. Seldes, *J. Org. Chem.*, 2000, **65**, 4482–4486.
6 Q. Ling, Y. Huang, Y. Zhou, Z. Cai, B. Xiong, Y. Zhang, L. Ma, X. Wang, X. Li and J. Li, *Bioorg. Med. Chem.*, 2008, **16**, 7399–7409.
7 L. M. Finkielsztein, A. M. Bruno, S. G. Renou and G. Y. M. Iglesias, *Bioorg. Med. Chem.*, 2006, **14**, 1863–1870.
8 T. G. Payne, Indaneacetic acid derivatives, *U.S. Patent* 4166131, issued August 28, 1979.
9 O. Laneuville, D. K. Breuer, D. L. Dewitt, T. Hla, C. D. Funk and L. W. Smith, *J. Pharmacol. Exp. Ther.*, 1994, **271**, 927–934.
10 R. G. Kurumbail, A. M. Stevens, J. K. Gierse, J. J. McDonald, A. R. Stegeman, J. Y. Pak, D. Gildehaus, J. M. Miyashiro, T. D. Penning, K. Seibert, P. C. Isakson and W. C. Stallings, *Nature*, 1996, **384**, 644–648.
11 K. C. Duggan, M. J. Walters, J. Musse, J. M. Harp, J. R. Kiefer, J. A. Oates and L. J. Marnett, *J. Biol. Chem.*, 2010, **285**, 34950–34959.
12 There may also be important metabolic difference between the neopentylene ring fusion of neoprofen compared to the isobutyl group of IBU, but such differences would not be apparent in our preliminary assays.
13 T. T. Hoang and G. B. Dudley, *Org. Lett.*, 2013, **15**, 4026–4029.
14 R. S. Jolly, G. Luedtke, D. Sheehan and T. Livinghouse, *J. Am. Chem. Soc.*, 1990, **112**, 4965–4966.
15 T. T. Hoang, M. Birepinte, N. M. Kramer and G. B. Dudley, *Org. Lett.*, 2016, **18**, 3470–3473.

