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Solution-phase parallel synthesis and screening of anti-tumor activities from fenbufen and ethacrynic acid libraries

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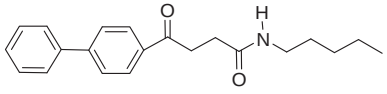
N-(4-Aminobutyl)-4-(biphenyl-4-yl)-4-oxobutanamide
Cell-based screening

ABSTRACT

The derivatives with fenbufen and ethacrynic acid core compounds was synthesized through a facial preparation of 1-amino-4-azidobutane. The subsequent coupling with 102 members of carboxylic acids afforded amide products. The in situ screening using colorimetric assay with 3-(4.5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide showed that fenbufen but not ethacrynic acid butyl amide members displayed the cytotoxicities to tumor cells substantially, including two human cell lines (MCF7 and A549) and two murine cell lines (C26 and TRAMP-C1). Three fenbufen analogs were found to have a good anti-tumor activity comparable to cisplatin.

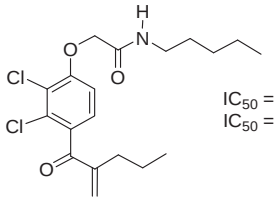
Parallel solution-phase synthesis (psps) has been used for lead generation and lead optimization of synthetic compounds.[1] The rapid purification or isolation of desired compounds from a reaction mixture represents a bottleneck in the synthetic procedure.[2] Thus, numerous approaches using solid-supported reagents and scavengers have been developed for isolation of library members.[3,4] On the other hand, in situ enzyme-based screening of the library mixture without prior purification has been developed by Wong and co-workers.[5] A number of potential compounds could be discovered by this approach.

By adopting a similar strategy, an in situ cell-based assay might provide another methodology for drug discovery. As an ongoing program of discovery for potent antitumor compounds, we were interested in developing a cell-based in situ screening protocol before studying their in vivo pharmacokinetics through an imaging modality in conjunction with radiofluoro-substituted (^{18}F) probe



butyl fenbufen

IC_{50} = 100 uM vs. A549
IC_{50} = 87 uM vs. MCF7



butyl ethacrynic acid

IC_{50} = 18 uM vs. A549
IC_{50} = 8 uM vs. MCF7

Abbreviations: A549 tumor, human type II epithelial cell line; MCF-7 tumor, solid human estrogen receptor positive breast carcinoma; TRAMP-C1 prostate cancer cell line, transgenic adenocarcinoma of the mouse prostate; C26 tumor, solid murine colon carcinoma; MTT assay, cell viability was measured with blue formazan that was metabolized from colorless 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial dehydrogenases which are active only in live cells; NSAIDs, non-steroid anti-inflammatory drugs.

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and positron emission tomography (PET). The initial concept has been performed through a cell-based screening of a minilibrary by psps via amide bond formation.[6] Two amide compounds, butylfenbufen and butyl ethacrynic acid, derived from fenbufen **3** and ethacrynic acid (EA) **8** coupled with butanamine showed cytotoxicities of IC_{50} of 87 and 18 μM to MCF7 and A549 tumor cells, respectively.[6] Furthermore, Lu and co-workers recently report that EA and its derivatives exhibit a selective toxicity to chronic lymphocytic leukemia by inhibition of the Wnt/βcatenin pathway.[7,8] Based on the above findings, it would be interesting to find out whether more potent analogs could be discovered through psps and the in situ cell-based screening. Thus, a desired member compound exhibiting inhibition concentration ranging in submicromolarity should be our ultimate goal. Therefore, fenbufen butanamine **5** and ethacrynic acid butanamine **10** emerged as the rational building templates for synthesis of libraries and in situ screening.

Since the structural features of the biphenyl ring moieties and the 1,2-dichloro-4-carbonyl benzene ring moieties are each required for the bioactivities of individual compound class, the appropriate structural diversity would be extended from the terminal end of the butyl chain. Thus, a bifunctional linker consisting of a protected amino group and a free amino group is prerequisite

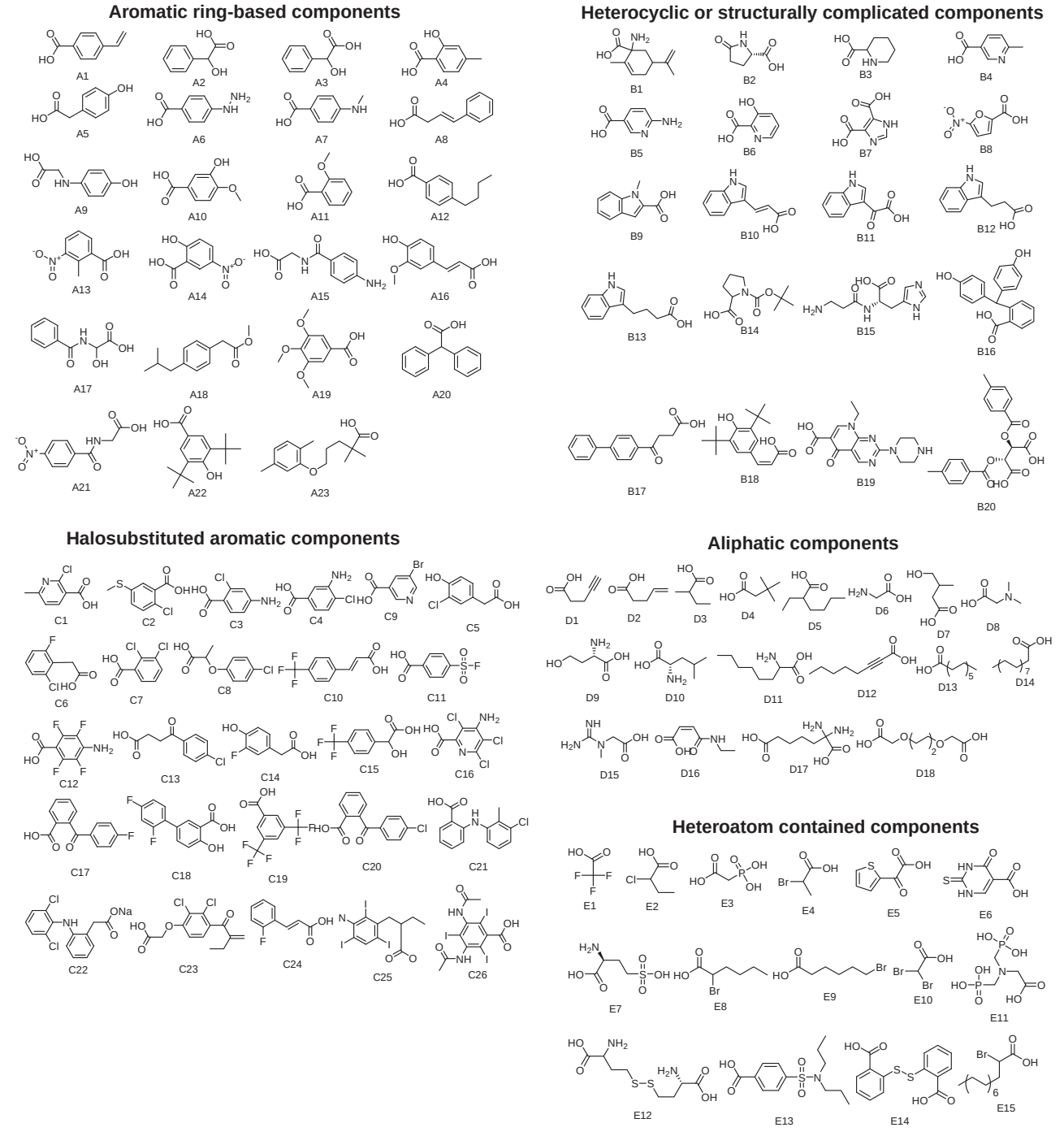
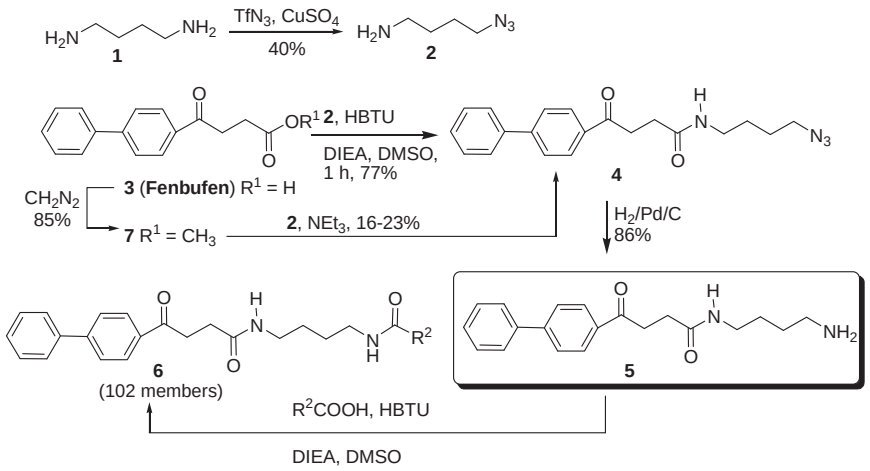
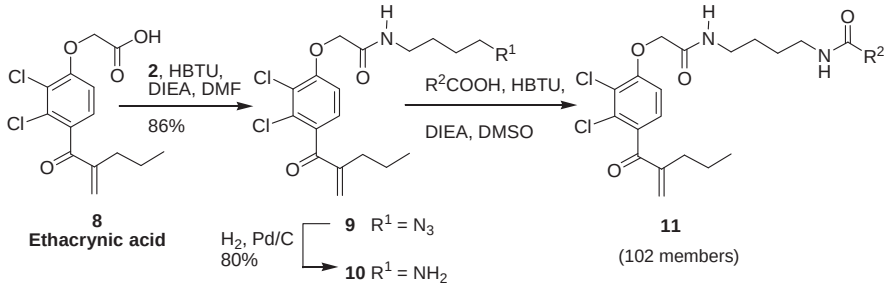


Figure 1. Carboxylic acid moieties for preparing the amide product.



Scheme 1. Reagents and conditions for preparation of libraries of butylfenbufen.



Scheme 2. Reagents and conditions for preparation of libraries of EA butylamide.

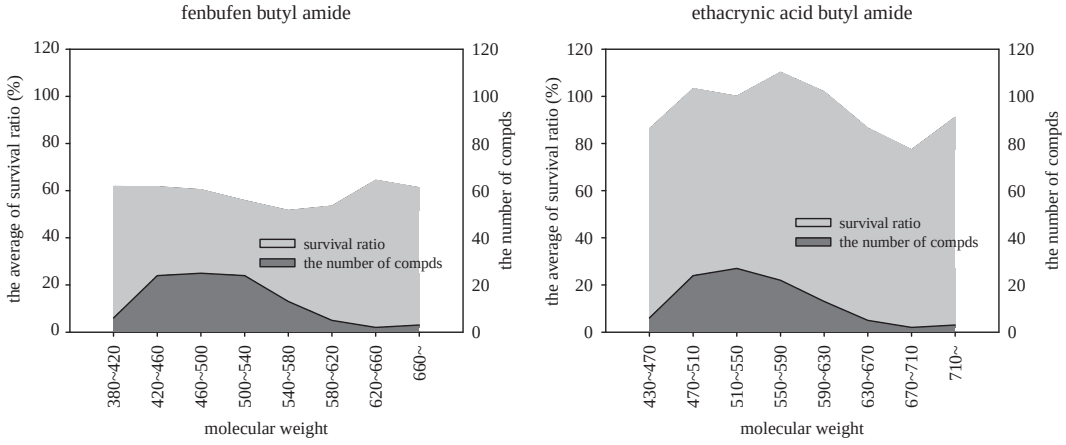


Figure 2. Molecular weight distribution of the amide members and the relevant average survival ratio from the four cell lines exerted whereby. (a) Data from fenbufen butyl amide. (b) Data for EA butylamide.

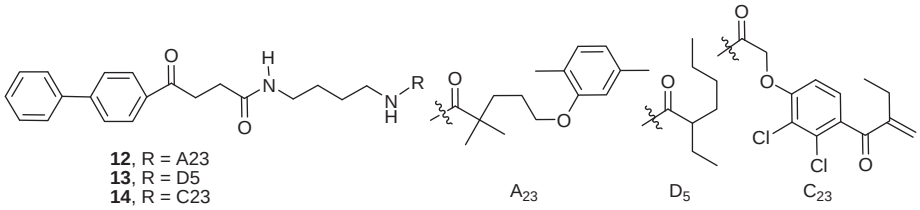


Figure 3. Structures of the potential compounds proven by validating experiment.

for synthesis of libraries. To this end, various synthetic approaches using either Boc or azido as the protecting groups have been reported.[9] Among them, azide as a protecting group particularly attracts our attention because of its easy preparation.[10] A number of synthetic routes toward 1-amino-4-azidobutane **2** have been reported, for example, reductive alkylation of an azide[9] or selective reduction of a diazidoalkane.[10] For shortening the synthetic route toward 1-amino-4-azidobutane **2**, here, we described an alternative synthesis of compound **2** from 1,4-diaminobutane **1** via azide transfer reaction under the catalysis of copper(II) ions (Scheme 1).[11] Its application toward the synthesis of the two core components of fenbufen **5** and EA **10** and subsequent coupling reaction were hereby described.

Instead of using column chromatography, compound **2** was obtained in an acceptable yield (40%) through partition. Though a significant amount of the byproduct, 1,4-diazido butane, was always accompanied in the mixture, the desired amide product **4** could be isolated by column chromatography in the subsequent reaction. Activation of the compound **3** by means of formation of an ester **7** to couple with compound **2** failed, however, to provide satisfactory yield of compound **4** at either room temperature or under reflux condition. Thus the usual activation condition using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium (HBTU) emerged as the proper choice.[12] After amide formation and subsequent reductive hydrogenation, the amine compound **5** obtained was readily served as a core compound for preparing the libraries of compound **6**.

However, purification of core compound **5** under the usual chromatographic condition was not an appropriate choice since very polar eluents such as ammonia (5%) in combination with MeOH and $CHCl_3$ would dissolve silica gel and make mess. By adopting a simple partition between CH_2Cl_2 and aqueous layer with pH value ranging from 10 to 11 (adjusted by a combination of $NaHCO_3$ (0.1 M) and NaOH (0.1 M)), the resultant organic salts and residual bases could be successfully removed. Likewise, a similar synthetic approach could be used for the preparation of EA amine **10** as the other core compound (Scheme 2). The two amino core compounds **5** and **10** were each obtained in 68–70% yields via two steps. The advantage of the current coupling method was attributed to a straightforward purification and thereby simplifies the overall synthesis. Coupling of both core compounds with 102 carboxylic acids provided the corresponding amide products (Fig. 1).

As inspired by a recent psps that showed a GAUSSIAN distribution for library member population versus MW sampling,[4a] we integrated the diagram of bioactivity profile against MW sampling (Fig. 2). As compared to EA butyl amide members, fenbufen butyl amide members, in general, exhibited cytotoxic activities against the four cell lines substantially. Compared to 100% average survival ratio by treatment with EA butyl amide members, the average survival ratio by fenbufen butyl amide members was down to 60%. Furthermore, the survival-ratio pattern showed a valley in the range of 540–620 D (Fig. 2a). Since the bioactivity trend could be biased with less sampling numbers, the present data would be statistically significant with sufficient samples with MW ranging from 420 to 580 D. A similar bioactivity pattern was also observed irrespective of the molecular weight sampling interval that was 50 or 30 D (Supplementary Figs. 3 and 4). Therefore, the pattern of bioactivity might be molecular weight-dependent. In contrast, EA butyl amide members showed two valleys at 430–470 and 670–710 D for bioactive regions (Fig. 2b). Whereas other MW sampling interval (MWSI) such as 30 D did not alter the biologic pattern, a significant change was found at the MWSI of 50 D that formed a big valley at 680–730 D. The dependency of bioactivity on MW is biased by MWSI. Thus, the current empirical manipulation needs further investigation.

To validate the apparent cytotoxicities induced by the crude members, an additional preparation and purification for the potential amides was critical. Hence, fenbufen butyl amide analogs with carboxylic acid moieties such as A23, D5, and D11 and EA butyl amide analogs with acid moieties for example, B15, B17 and E15 deserved a further examination. The self-coupling of acids B15 and D11, as encountered during library preparation, however, discouraged further attempt. Besides, since both EA butyl amides B17 and E15 displayed comparable bioactivity profiles, the B17 amide was chosen for further analysis. Thus, three potential compounds **12**–**14** were prepared separately and tested for MTT assays (Fig. 3).

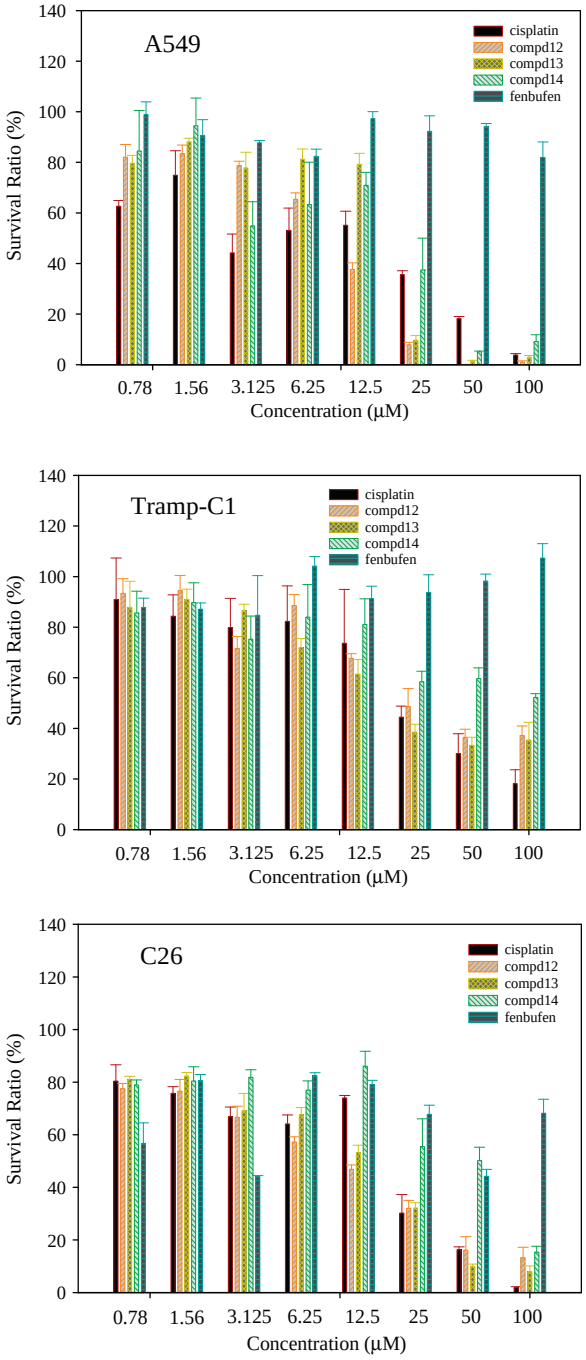


Figure 4. Effects of three potential compounds on a survival ratio of A549, TRAMP-C1, and C26 cells. Cisplatin and fenbufen were used as positive control and negative control, respectively. Error bar was calculated from the measurements in triplicate.

Table 1
Concentration determined for half surviving cells by MTT assay

Tumor cells	IC_{50} value (μM)				
	Cisplatin	12	13	14	Fenbufen
A549	6[a]	9	17	19	>100
MCF7	25[b]	2	1	5	>50
TRAMP-C1	22	40	18	80	>100
C26	17	10	13	35	>100

[a] IC_{50} = 1.3–11 μM.[6,14]
[b] IC_{50} = 14–31 μM.[6,15]

The results confirmed that compound **12** had an equivalent cytotoxicity of cisplatin against A549 cells (Fig. 4, Table 1). Interestingly, compound **14** was not potent although it retained both structural characteristics of fenbufen and ethacrynic acid.

The probable cause of lacking improvement on the bioactivity profile by the ethacrynic acid members might be due to the inappropriateness of butyl linkage. On the other hand, Lipinski's rule of 5 might provide the other reasonable rationale.[13] The rule predicts that the drug-like compounds have less than 5H-bond donors and 10 H-bond acceptors and the molecular weight is less than 500 D. Since the core of EA members contained 7H-bond acceptors, its coupling products would contain more than 10H-bond acceptors, such as the coupling products from B17, E10 and E15.

In brief, the present parallel synthesis and in situ screening of the amide library through 4-azido butanamine as a linker allowed a discovery of the fenbufen analogs **12–14** with various anti-tumor activities, as compared to cisplatin. In addition, in view of the anti-tumor potencies accompanied with the initial role as NSAIDs, the biologic roles played by the fenbufen analogs warrant further study.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.01.068.

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