Hunting the Emesis and Efficacy Targets of PDE4 Inhibitors: Identification of the Photoaffinity Probe 8-(3-Azidophenyl)-6-[(4-iodo-1*H*-1-imidazolyl)methyl]quinoline (APIIMQ)

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Received February 16, 2000

Introduction. Type 4 cAMP-specific phosphodiesterase (PDE4) is an enzyme responsible for the hydrolysis of the second messenger c-AMP to AMP in many cell types.[1] Inhibition of this enzyme can significantly increase the intracellular c-AMP concentration, leading to major alterations in cell biochemistry and function. In particular, some inflammatory processes can be attenuated with PDE4 inhibitors. For example, LPS-stimulated TNF-α release in human blood mononuclear cells can be blocked with PDE4 inhibitors.[2] Antigen-induced bronchospasm is another pharmacological event that can be attenuated using PDE4 inhibitors.[3] Because both inflammation and bronchoconstriction are major factors in asthma, PDE4 is a promising therapeutic target for this common and serious disease. One of the major issues with the development of PDE4 inhibitors, however, has been the side effect of emesis observed for several prototypical compounds.[4] More recently, it has become clear that some PDE4 inhibitors are less emetic than others while possessing the same potency.[4,5] For several years, efforts to improve the therapeutic window of PDE4 inhibitors have involved the identification of compounds that appeared to be more potent for inhibiting the enzyme activity and less potent on the high-affinity rolipram-binding site of the enzyme.[1] Recently, however, it has been shown that the high-affinity rolipram-binding site of the enzyme is simply the cofactor-bound active site on the holoenzyme. The conformational difference between the PDE4 apoenzyme and the holoenzyme is responsible for the differential binding of inhibitors.[5d,e] Other recent efforts have been directed toward the identification of isozyme-selective compounds.[1a]

In this communication, we introduce a new approach to improving the therapeutic window of PDE4 inhibitors, which is aimed at the identification of the specific targets for emesis and efficacy. To this end we have prepared an emetic, efficacious, and competitive PDE4 inhibitor (**23**, APIIMQ) capable of covalently tagging its biological targets upon photoactivation. This provides the possibility of identifying the emesis and efficacy targets of PDE4 inhibitors. To our knowledge, this is the first reported example of the preparation of a highly emetic and efficacious PDE4 photoaffinity probe.

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Chemistry. Rolipram (**1**) is commercially available. The compounds **2**, **3** (KF17625), **4** (KF18280), and **5** shown in Schemes 1 and 2 and Table 1, were prepared as described in the literature.[6] Alkylation of compounds **1** and **3** with NaH and **2** in DMF afforded compounds **6** and **7** (Scheme 1).

Reduction of the nitro group in compound **5** resulted in the aniline **8** (Scheme 2), which was converted to the aryl azide **9** by diazotization and displacement with azide. The 3-azido-6-iodo compound **10** was similarly prepared, except that the aniline **8** was iodinated with ICl prior to its conversion to the azide.

Compounds **15**, **16**, and **18** were prepared using chemistry similar to that described in the literature[6b] for compounds in the class of **5**, except that the iodo and azido substituents were introduced by modification of an aniline, a nitro group, or a carboxylic acid (Schemes 3 and 4).

Compounds **20** and **23** were produced using similar chemistry except that the imidazole and iodoimidazole substituents were introduced by displacement of the bromomethylquinolines **19** and **21** (prepared as in the literature[6b]) with imidazole and iodoimidazole, respectively (Scheme 5). The displacement with 5-iodoimidazole yielded the 4-iodo **22a** as the major product and the 5-iodo **22b** as the minor product. The regiochemistry of the two iodoimidazoles **22a**,**b** was determined using NOE experiments. In all the azide compound sytheses, care was taken to design the synthesis such that the light-sensitive azido group was introduced in the last step.

Conversion of ^{127}I-**23** to ^{125}I-**23** was accomplished by palladium-catalyzed tributylstannylation of the iodoimidazole to give compound **24** followed by radioiodination with Na^{125}I and chloramine-T (Scheme 6).

Recombinant Human PDE4. PDE4A^{248} was expressed as a GST-fusion protein from SF9 cells and purified to near homogeneity as previously described.[5b]

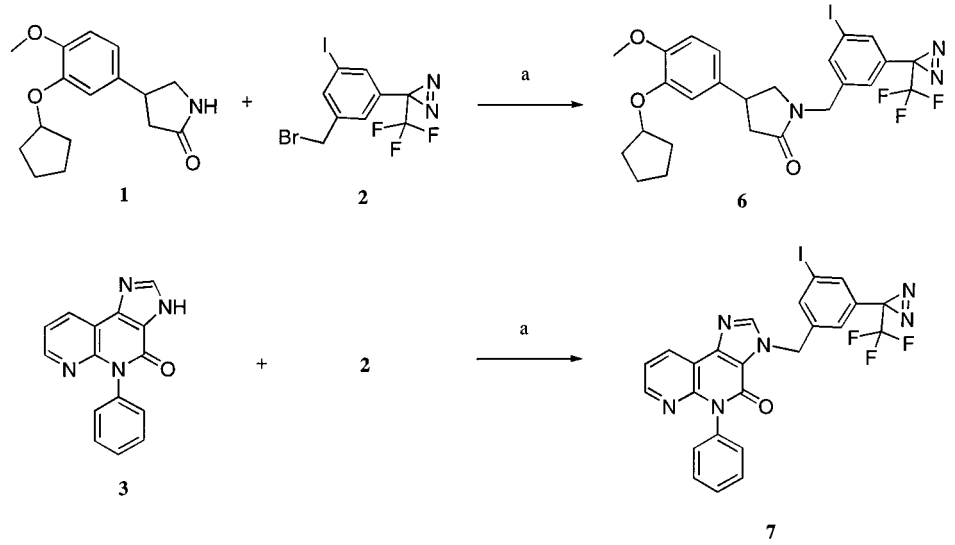
PDE Assay. The compound's potency on inhibiting PDE4 catalysis was determined using purified GST-PDE4A^{248} as previously described.[5d]

Results and Discussion. A number of different functional groups have been described in the literature for photolabeling target proteins. These include the aryl azide, the trifluoromethyl diazerene, and the benzophenone moiety.[7] One of the most common radioisotopes with a high specific activity, leading to a high detection sensitivity, incorporated in photoaffinity probes of this type is ^{125}I. Because these groups have significant spatial requirements, it is important to identify positions on the inhibitor of interest where the groups are tolerated. To obtain a compound containing these groups that is also emetic and effective in our functional model, we chose to work on several different classes of known emetic PDE4 inhibitors.

The first class of inhibitors we considered was that based on the prototypical compound rolipram (**1**), found to be emetic in both animal models and humans.[4] Incorporation of an *N*-(3-iodo-5-trifluoromethyldiazerenobenzyl) substituent on rolipram (Scheme 1) led to a 10-fold loss in potency (see **6** in Table 1). Compound **6**

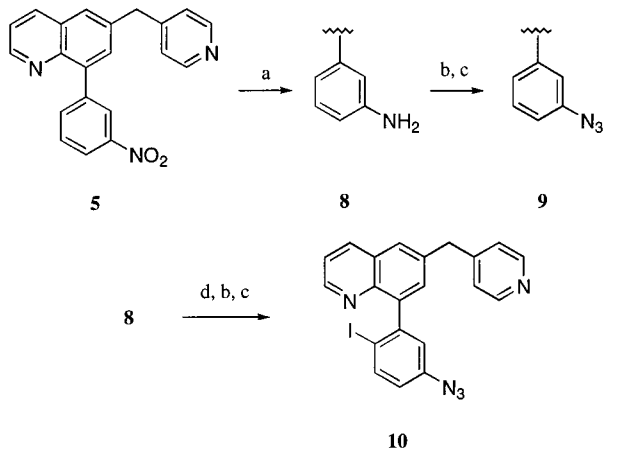
10.1021/jm000065c CCC: $19.00
Published on Web 09/30/2000

Scheme 1[a]



[a] Reagents and conditions: (a) NaH, DMF, rt, 1 h.

Scheme 2[a]



[a] Reagents and conditions: (a) Fe, NH_4Cl, EtOH, H_2O, reflux; (b) AcOH, $NaNO_2$, H_2O, 0 °C, 15 min; (c) NaN_3, 0−25 °C, 2 h; (d) ICl, $CaCO_3$, MeOH, H_2O, rt, 2 h.

was also not emetic at a dose 100-fold higher than the emetic threshold of rolipram in the ferret.

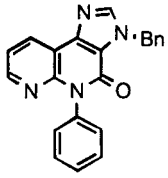
Another highly potent and emetic PDE4 inhibitor is compound **4** (KF18280).[6a] The same substituent as that used on rolipram to give compound **7** (Scheme 1) also resulted in a considerable loss in potency (Table 1).

The most potent class of inhibitors we have considered is the arylquinoline, as typified by compound **5**.[6b] Substitution of the nitro group for an azido gave compound **9** (Scheme 2). This compound not only retains high potency (0.2 nM) but also has a very low emetic threshold. The next requirement was to identify a suitable position to locate the iodine substituent. Simple substitution on the ring para to the azido by iodinating the aniline intermediate **8** (Scheme 2**)** resulted in a loss in potency (compound **10**). Because the potency of these compounds is very sensitive to substitution on the 8-phenyl, we next chose to place a relatively small group only in the meta position, which tolerates such groups. Another position that tolerates a variety of substituents is the 6-position on the quinoline. We therefore introduced the azido and iodo substituents at either of these

Table 1. PDE4A Potency and Emetic Threshold in Ferrets

		emetic threshold in ferret[a,b]	
compd	PDE4A[a] IC_{50} (nM)	dose (mg/kg, po)	plasma concn (μM)
1	4 ± 2	0.1 ± 0.05	<0.3[c]
6	35 ± 4	>10[d]	7.0 ± 0.2
4[e]	0.6 ± 0.1	1 ± 0.5	0.2 ± 0.1
7	250 ± 20	>10[d]	
5	0.07 ± 0.05	0.1 ± 0.05	
9	0.2 ± 0.1	0.1 ± 0.05	
10	3.3 ± 0.5	>30[d]	16.0 ± 0.2
15	15 ± 5	>10[d]	0.8 ± 0.1
16	28 ± 5	>10[d]	<0.3[c]
18	2.7 ± 0.2	>10[d]	<0.3[c]
20	9 ± 3	0.1 ± 0.05	
23	0.4 ± 0.1	0.1 ± 0.05	<0.2[c]

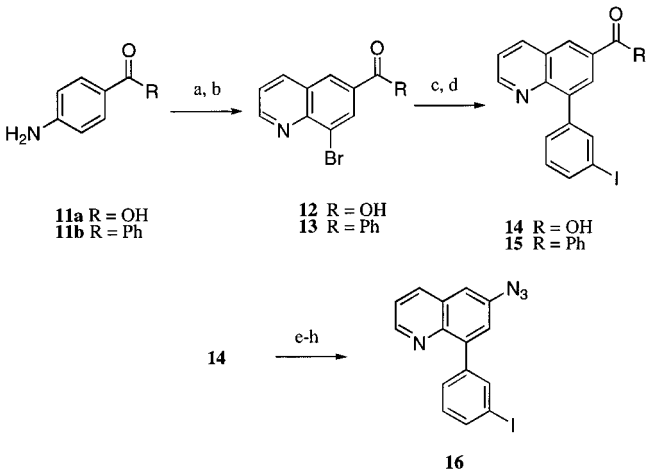
[a] $N \geq 2$. [b] See ref 8 for method. [c] Limit of detection. [d] No emesis was observed at the dose specified. [e] Structure of **4**:



two positions. The resulting compounds **16** and **18** (Schemes 3 and 4) retain considerable potency (28 and 2.7 nM, respectively) but, unfortunately, are not emetic at 10 mg/kg in the ferret. Introduction of a benzoyl in place of the azido in **16** resulted in a compound with modest potency (see **15** in Scheme 3) and still not emetic at 10 mg/kg in the ferret.

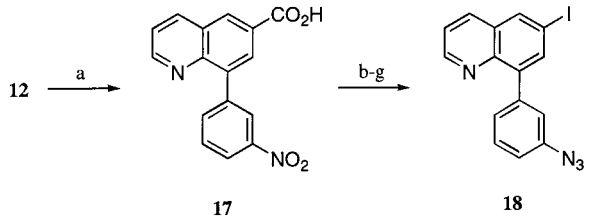
The fact that compounds **16** and **18** were not detectable in the plasma when dosed in the ferret suggested that the high lipophilicity and poor aqueous solubility (leading to poor pharmacokinetics) may be responsible for the lack of emesis from these compounds. To introduce polarity into the molecule, we took note of the fact that the highly polar imidazole-substituted compound **20** (Scheme 5) has a very low emetic threshold. Substitution of this compound with an iodo on the imidazole ring and an azido on the phenyl ring afforded compound **23** (Scheme 5), which is quite potent on PDE4A (0.4 nM) and is also emetic in the ferret at very low doses (0.1 mg/kg). Compound **23** is also potent in our human whole blood assay for the inhibition of LPS-

Scheme 3[a]



[a] Reagents and conditions: (a) NBS, DMF, rt, 18 h; (b) glycerol, H_2SO_4, H_2O, sodium 3-nitrobenzenesulfonate, 150 °C, 30 min; (c) 3-aminobenzeneboronic acid, $Pd(PPd_3)_4$, $NaCO_3$, DME, reflux, 5 h; (d) HI, $NaNO_2$, KI, PEG-200, CH_2Cl_2, 0−25 °C, 18 h; (e) diphenylphosphoryl azide, Et_3N, *t*-BuOH, reflux, 18 h; (f) TFA, CH_2Cl_2, rt, 3 h; (g) AcOH, $NaNO_2$, H_2O, 0 °C, 15 min; (h) NaN_3, 0−25 °C, 2 h.

Scheme 4[a]

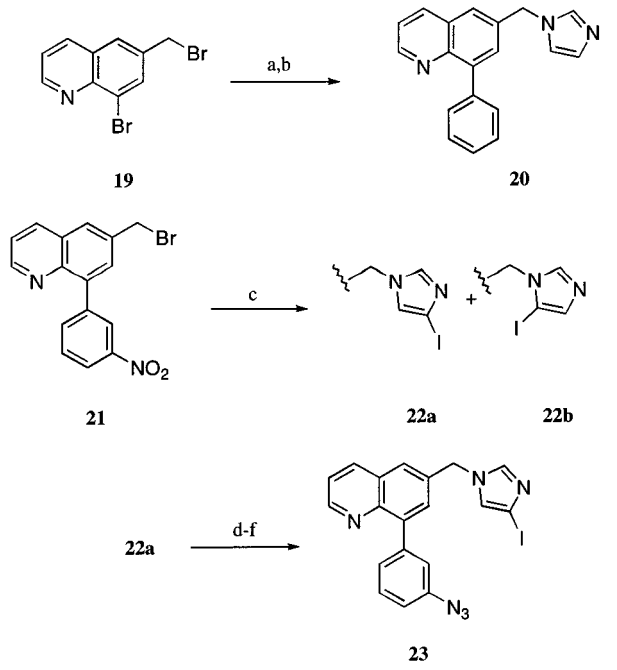


[a] Reagents and conditions: (a) 3-nitrobenzeneboronic acid, $Pd(PPd_3)_4$, $NaCO_3$, DME, reflux, 6 h; (b) diphenylphosphoryl azide, Et_3N, *t*-BuOH, reflux, 10 h; (c) TFA, CH_2Cl_2, rt, 30 min; (d) HI, $NaNO_2$, KI, PEG-200, CH_2Cl_2, 0−25 °C, 2 h; (e) Fe, NH_4Cl, EtOH, H_2O, reflux, 1 h; (f) AcOH, $NaNO_2$, H_2O, 0 °C, 30 min; (g) NaN_3, rt, 3 h.

induced TNF-α ($IC_{50} = 2 \pm 1$ μM)[2] and in our guinea pig ovalbumin-induced bronchoconstriction model ($ED_{50} = 0.3 \pm 0.2$ mg/kg).[3b] To evaluate the protein labeling ability of compound **23**, we converted it to the ^{125}I version (Scheme 6).

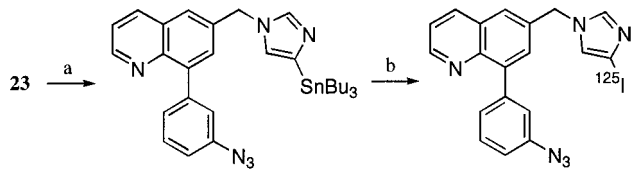
^{125}I-**23** specifically labels recombinant PDE4 in the presence of other cellular proteins under photolysis conditions. Shown in Figure 1 is the SDS gel radioactivity image of 100 μg of SF9 cell lysate proteins spiked with 5 ng of GST-PDE4A^{248} after photolysis with 1 nM ^{125}I-**23**. A band with a mobility of about 110 kDa that comigrated with pure GST-PDE4A^{248} on Commassie stain (data not shown) was specifically labeled after photolysis (inside circle in lane 3). This band was absent in the null cell lysate. No labeling was observed in absence of light (data not shown) indicating that the formation of the covalent adduct was photochemically specific. An active-site-directed potent PDE4 inhibitor, CDP-840[5b] (10 μM, $K_i \sim 4$ nM), specifically competed with the photolabeling of ^{125}I-**23** (lane 4), demonstrating that **23** is an active-site-directed-specific photoprobe. The probe is also highly sensitive and specific because 5 ng of GST-PDE4A^{248} was readily identified in the presence of 100 μg of total cellular proteins. The probe's high detection sensitivity and its high emetic potency in vivo suggest that **23** will be a powerful tool in studying the distribution of its high-affinity targets in specific tissues and in associating these targets with its biological responses such as efficacy and emesis. Further

Scheme 5[a]



[a] Reagents and conditions: (a) imidazole, THF, reflux, 3 h; (b) phenylboronic acid, $Pd(PPh_3)_4$, $NaCO_3$, DME, 75 °C, 18 h; (c) 5-iodoimidazole, Cs_2CO_3, DMF, rt, 18 h; (d) Fe, NH_4Cl, THF, EtOH, H_2O, 70 °C, 40 min; (e) AcOH, $NaNO_2$, H_2O, 0 °C, 25 min; (f) NaN_3, 0−25 °C, 30 min.

Scheme 6[a]



[a] Reagents and conditions: (a) $Bu_3SnSnBu_3$, $Pd(dba)_2$, Ph_3As, DMF, 135 °C, 1 h; (b) Na^{125}I, chloramine-T, DMF, H_2O, rt, 1 h.

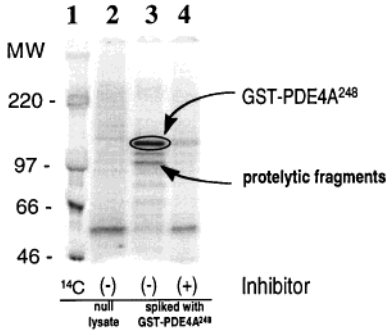


Figure 1. Radioactivity image of the 100 μg of cell lysate spiked with 5 ng of GST-PDE4A^{248} photolabeled with 1 nM ^{125}I-**23**: lane 1, ^{14}C-labeled molecular weight marker; lane 2, null cell lysate; lane 3, cell lysate spiked with 5 ng of GST-PDE4A^{248}; lane 4, cell lysate spiked with 5 ng of GST-PDE4A^{248} in the presence of 10 μM CDP-840. The two weaker bands immediately below the intensely labeled GST-PDE4A^{248} are protelytic fragments derived from GST-PDE4A^{248} as judging from their positive immunoreactivity with a PDE4-specific antibody.

characterization of the probe in addition to its use in the identification of biological targets in various tissue types is underway.

Conclusion. In conclusion, we have identified compound **23** (APIIMQ) as a potent, efficacious, and competitive radiolabeled inhibitor of PDE4, which covalently labels the enzyme upon light activation. Compound **23** also has a very low emetic threshold (0.1 mg/kg, po, in ferrets). This probe should be very useful for the identification of the respective targets through which PDE4 inhibitors cause emesis and also produce their efficacy.

Acknowledgment. We are grateful to Dr. Michel Gallant and Dr. Cameron Black for kindly providing samples of compounds **2**−**4**.

Supporting Information Available: Synthetic chemistry methods, biological procedures, and chemical characterization data for compounds **22**−**24** is available free of charge via the Internet at http://pubs.acs.org.

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