(12) STANDARD PATENT (11) Application No. **AU 2017202419 B2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
Pharmaceutical compounds for use in the therapy of clostridium difficile infection

(51) International Patent Classification(s)
C07F 9/117 (2006.01)

(21) Application No: **2017202419** (22) Date of Filing: **2017.04.12**

(43) Publication Date: **2017.05.11**
(43) Publication Journal Date: **2017.05.11**
(44) Accepted Journal Date: **2017.08.31**

(62) Divisional of:
2012314932

(71) Applicant(s)
ETH Zurich

(72) Inventor(s)
Castagner, Bastien;Leroux, Jean-Christophe;Ivarsson, Mattias;Schneider, Gisbert;Pratsinis, Anna

(74) Agent / Attorney
Baldwins Intellectual Property, 16 Chisholm Street, North Ryde, NSW, 2113, AU

(56) Related Art
WO 2012/138963 A1

The invention relates to a compound described by a general formula (I), wherein each X independently can be OPO_3^{2-}, $OPSO_2^{2-}$ or OSO^{3-}; R^1 comprises a solubility function such as a polyethylene glycol moiety and each X independently can be OPO_3^{2-}, $OPSO_2^{2-}$, or OSO^{3-}; and Z is an alkyl chain comprising 1 to 3 carbon and/or hetero atoms. Dosage forms and methods of treating or preventing *Clostridium difficile* infections with the aforementioned cyclic polyols are also provided.

Pharmaceutical Compounds for use in the therapy of *Clostridium difficile* Infection

Description

The present application is a divisional application filed out of AU 2012314932.

The present invention relates to enteric activators of *Clostridium difficile* toxin, particularly polyphosphate derivatives, polysulfate derivatives or mixed polyphosphate/sulphate derivatives of six-membered cyclic polyols.

Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of the common general knowledge of the field.

Clostridium difficile is a species of Gram-positive bacteria that causes severe diarrhoea in human patients. *C. difficile* infection (CDI) typically affects patients under antibiotic treatment since the bacterium is only able to colonize the colon of patients with depleted bacterial flora. The emergence of antibiotic-resistant strains of *C. difficile* causes increasingly severe morbidity and mortality due to the spread of new, more virulent strains, with recent outbreaks in North America and Europe.

C. difficile asymptomatically colonizes 2-5% of the human adult population. The bacteria form spores, which are difficult to neutralize by common methods of disinfection. As a result, *C. difficile* infections are a common result of prolonged stays in hospitals; the pathogen is considered the leading cause of hospital-associated diarrhoea in the USA.

Current therapy of choice is oral application of metronidazole or, in case of failure of the former, vancomycin. Since clinical symptoms of CDI are caused by two toxic proteins secreted by *C. difficile* in the colon, rather than by the presence of the bacteria itself, efforts have been made recently to target these toxins (e.g. employing polymeric binders), but have so far failed in clinical trials.

C. difficile enterotoxin (toxin A, TcdA) and cytotoxin (toxin B, TcdB) are the main contributors to the symptoms of disease (for a toxin biology review, see Voth and Ballard, *Clinical Microbiology Reviews* **2005**, 18, 247-263). In brief, both toxins are composed of four domains, a first domain mediating the attachment of the toxin to cells; a second one facilitating translocation into the cytosol; a third domain causing the cleavage of the toxic domain by autoproteolysis, and finally the toxic domain or "warhead" itself, which causes the physiological effects of the toxin in the affected cell.

Reineke *et al.* (*Nature* **2007**, 446, 415) identified *myo*-inositol hexakisphosphate (IP6) as the natural trigger of TcdA/TcdB autoprocessing in the cell cytosol. Egerer *et al.* (*PLoS Pathog.* **2010**, *6*, e1000942) and Shen *et al.* (*Nat. Struct. Mol. Biol.* **2011**, *18*, 364) suggested targeting the IP6-induced autoprocessing mechanism as a means of therapeutic intervention against toxin-mediated pathogenicity.

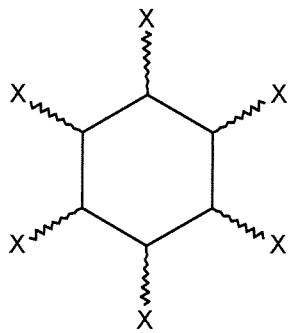
2017202419 12 Apr 2017

Kreimeyer *et al.* suggested using IP6 pharmaceutically to intervene in CDI (*Naunyn-Schmiedeberg's Arch. Pharmacol.* **2011**, *383*, 253). However, this approach is not feasible as the presence of high calcium concentrations in the colon precipitates IP6 and prevents it from being active.

Thus, the objective of the present invention is to provide improved treatment options for patients suffering from CDI. This objective is attained by the subject-matter of the independent claims.

The invention is based on a novel design of small-molecule analogues of IP6 that are provided as an oral therapy to trigger the cleavage of the toxin in the colon lumen, thereby detaching the warhead before it reaches its destination, and rendering it harmless. Since IP6 itself cannot be used for this purpose because it is not soluble at the high calcium concentrations found in the colon lumen, the present invention provides new analogues of IP6 with improved solubility.

According to a first aspect of the invention, a pharmaceutical compound characterized by a general formula (1) is provided,

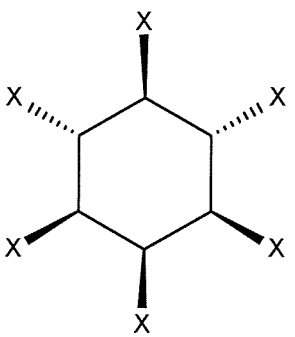


(1)

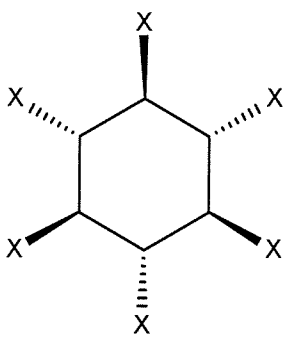
wherein each X independently is selected from OPO_3^{2-}, $OPSO_2^{2-}$, or OSO_3^-, with the proviso that not all X are OPO_3^{2-} and not all X are OSO_3^-.

According to another aspect of the invention, the compound characterized in the previous paragraph by formula (1) is provided for use as a medicament, particularly for use in the prevention or therapy of infections by *Clostridium dificile*.

In some embodiments, the compound according to this first aspect of the invention is characterized by a general formula (1a) or (1b), wherein X has the meaning outlined above:

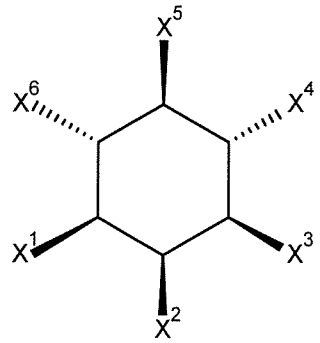


(1a) (*myo*)

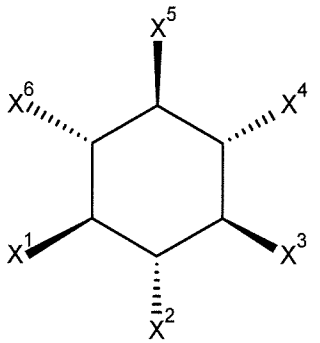


(1b) (*scyllo*)

In some embodiments, the compound according to this first aspect of the invention is characterized by the general formula (2a) or (2b),



(2a)



(2b)

wherein

a) X^2 is OSO_3^-, and X^1, X^3, X^4, X^5 and X^6 are independently selected from OPO_3^{2-}, $OPSO_2^{2-}$ or OSO_3^-;

b) X^1, X^3 and X^5 are OPO_3^{2-} and X^2, X^4 and X^6 are OSO_3^- (Compound 2a-b or 2b-b),

c) X^1, X^3 and X^5 are OSO_3^- and X^2, X^4 and X^6 are OPO_3^{2-} (Compound 2a-c or 2b-c),

d) X^4, X^5 and X^6 are OSO_3^- and X^1, X^2 and X^3 are OPO_3^{2-} (Compound 2a-d or 2b-d),

e) X^4, X^5 and X^6 are OPO_3^{2-} and X^1, X^2 and X^3 are OSO_3^- (Compound 2a-e or 2b-e),

f) X^2 and X^5 are OPO_3^{2-} and X^1, X^3, X^4, and X^6 are OSO_3^- (Compound 2a-f or 2b-f),

g) X^2 and X^5 are OSO_3^- and X^1, X^3, X^4, and X^6 are OPO_3^{2-} (Compound 2a-g or 2b-g),

h) X^2 and X^3 are OPO_3^{2-} and X^1, X^4, X^5, and X^6 are OSO_3^- (Compound 2a-h or 2b-h), or

i) X^2 and X^3 are OSO_3^- and X^1, X^4, X^5, and X^6 are OPO_3^{2-} (Compound 2a-i or 2b-i).

The compounds defined above can be synthesized according to standard methods. The synthesis of compound 2a-b is described in the examples of the present invention.

According to another aspect of the invention, a compound according to any of the above aspects of the invention, in the broadest definition given, or as specified in any of the embodiments, is provided for use as a medicament.

According to yet another aspect of the invention, a compound according to any of the above aspects of the invention, in the broadest definition given, or as specified in any of the embodiments, is provided for use in the treatment or prevention of *C. difficile* infection.

A compound according to the invention may be given to a patient already diagnosed with CDI, or to a patient being suspected of suffering from CDI. Alternatively, the compound may be used as a prophylactic for patients that are at risk of contracting the infection, such as patients under antibiotic treatment in hospital settings. The compounds according to the invention are simple to synthesize, resistant to degradation in the gastro-intestinal tract and unlikely to be absorbed into the bloodstream, thus avoiding potential side effects. The compounds according to the invention do not need to penetrate mammalian or bacterial membranes to be active, which makes them more effective *in vivo*.

In addition, the compounds according to the invention are unlikely to exert selective pressure on the bacteria and therefore avoid problems related to resistance.

According to yet another aspect of the invention, a pharmaceutical composition for use in a method for the prevention or treatment of *C. difficile* infection is provided, comprising a compound according to any of the above aspects of the invention.

Preferred pharmaceutical compositions comprise from approximately 1% to approximately 95% active ingredient, preferably from approximately 20% to approximately 90% active ingredient.

A pharmaceutical composition according to the above aspects of the invention can be administered alone or in combination with one or more other therapeutic agents. A combination therapy may take the form of fixed combinations of the compound of the invention and one or more other antibiotic agents. Administration may be staggered; alternatively drugs may be given independently of one another, or as a fixed combination.

According to a preferred embodiment, a pharmaceutical composition comprises a compound of the invention according to any of the above aspects of the invention, and additionally metronidazole, vancomycin and/or fidaxomicin.

According to yet another aspect of the invention, a dosage form is provided comprising a compound according to any of the above aspects of the invention. A peroral formulation, particularly a tablet, syrup, solution, capsule or powder is preferred.

According to a preferred embodiment, such a dosage form additionally comprises an antibiotically active compound, such as (by way of non-limiting example) metronidazole, vancomycin or fidaxomicin.

In certain embodiments, the dosage form is a tablet, capsule, solution, powder or syrup.

According to yet another aspect of the invention a treatment regime is provided for the prevention and treatment of CDI, comprising the administration of a compound according to the invention. Administration may be effected by any of the means described herein.

Also within the scope of the present invention is a method for the prevention or treatment of CDI, comprising the administration a compound according to the invention to a subject in need thereof.

Similarly, a compound according to the invention is provided for the manufacture of a medicament for the prevention and treatment of CDI. Medicaments according to the invention are manufactured by methods known in the art, especially by conventional mixing, coating, granulating, dissolving or lyophilizing.

Wherever alternatives for single features are laid out herein as “embodiments”, it is to be understood that such alternatives may be combined freely to form discrete embodiments of the entire molecule provided as such or for use in a method or medical indication herein.

Short description of the figures

Fig. 1 shows the synthesis of compound (2a-b).

Fig. 2 shows the concentration dependence of cleavage of TcdB cysteine protease domain in the presence of 10 mM Ca^{2+} for activator compound (2a-b).

Examples

1. Determination of EC50 in presence of 10 mM Ca^{2+}

The compound to be tested was added to a recombinant His-tagged cysteine protease domain of *C. difficile* toxin B of SEQ ID 1 in presence of 10 mM Ca^{2+} in 100 mM Tris pH7.4 and incubated for 2 h at 37°C. Cleaved protein fragments were separated by SDS-PAGE and visualized by Coomassie staining. The extent of cleavage quantified from protein band intensities using the ImageJ software package. Signals were normalized to cleavage of positive and negative controls.

2. Comparison of cleavage kinetics

The compound to be tested was added to the His-tagged cysteine protease domain of *C. difficile* toxin B (same sequence as given above) in presence of 10 mM Ca^{2+} in 100 mM Tris pH 7.4 and incubated for 24 h at 37°C, with aliquots removed at regular intervals. Cleaved protein fragments were separated, visualized and analyzed as indicated above.

3. Synthesis of compound (2a-b)

Compound **F**: A solution of 2,4,6-tri-*O*-(4-methoxybenzyl)-*myo*-inositol (**E**) [D. Lampe, C. Liu, B. V. L. Potter, *J. Med. Chem.* **1994**, *37*, 907] (0.541 g, 1 mmol, 1 eq.) in dry CH_2Cl_2 (20 ml, 0.05 m) under an atmosphere of nitrogen was treated with tetrazole in acetonitrile 0.45 m (20.0 ml, 9.0 mmol, 9 eq.) and *o*-xylylene-*N,N*-diethylphosphoramidite (6 mmol, 1.44 g, 6 eq.). The reaction mixture was stirred at r.t. for 2 days. A solution of *m*CPBA (12 mmol, 2.07 g, 12 eq.) dried over Na_2SO_4 was added at -10 °C and the reaction mixture was stirred at r.t. for an additional 45 min. The mixture was then diluted in EtOAc, washed with a saturated solution of aqueous $NaHCO_3$ and with brine. The organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash chromatography (SiO_2, CH_2Cl_2/MeOH gradually from 0 % to 4 %, three times) afforded 2,4,6-tri-*O*-(4-methoxybenzyl)-1,3,5-tri-*O*-(*o*-xylylenephospho)*myo*-inositol (**F**) as a white solid (98 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.28-7.36 (12H, m), 7.19-7.22 (2H, m), 7.12-7.17 (4H, m), 6.86 (2H, d, *J* 8.5 Hz), 6.71 (4H, d, *J* 8.5 Hz), 5.25 (1H, d, *J* 13.6 Hz), 5.21 (1H, d, *J* 13.6 Hz), 5.15 (1H, d, *J* 13.7 Hz), 5.11 (1H, d, *J* 13.7 Hz), 4.91-5.08 (8H, m), 4.83-4.89 (4H, m), 4.69-4.61 (3H, m), 4.57 (1H, q, *J* 9.2 Hz), 4.38 (2H, ddd, *J* 2.4, 8.1, 9.5 Hz), 4.10 (2H, t, *J* 9.5 Hz), 3.78 (3H, s), 3.72 (6H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 159.3, 159.1, 135.4, 135.3, 135.2, 130.8, 130.3, 129.7, 129.6, 129.2, 129.13, 129.12, 129.0, 128.9, 128.6, 113.74, 113.57, 80.6 (d, J_{CP} 6.0 Hz), 78.1 (dd, J_{CP} 6.9, 3.2 Hz) 77.6, 77.1 (m), 76.0, 74.9, 68.8, 68.70, 68.68, 68.62, 68.34, 68.28, 55.4, 55.3; ^{31}P NMR (160 MHz, ^{1}H-decoupled, $CDCl_3$) δ (ppm) 1.10, -1.32.

Compound **G**: Compound **F** (97 mg, 0.089 mmol) was dissolved in 1 mL dichloromethane. Added 6 mL of a 5:1 mixture of trifluoroacetic acid-water. Stirred 25 min and then diluted with 10 mL toluene and concentrated under vacuum. The resulting residue was triturated with hexane and

dichloromethane and then dried under high vacuum. Yielded 68 mg of crude compound **G** that was used directly in the next step.

Compound **H**: Compound **G** (39 mg, 0.054 mmol) was dissolved in 3 mL DMF and $SO_3 \cdot Et_3N$ (195 mg, 1.07 mmol) was added. The solution was stirred overnight at 50°C and concentrated on a rotavap. The residue was dissolved in 6 mL water, filtered and loaded on three Vac 6cc 1g tC18 Sep-Pak cartridges (Waters). The columns were eluted with a gradient from 0-40% MeOH/H_2O. Yielded 32 mg of **H**. ^1H NMR (400 MHz; MeOD): δ 7.45-7.40 (m, 4H), 7.39-7.33 (m, 4H), 7.28-7.24 (m, 2H), 7.21-7.19 (m, 2H), 5.69-5.60 (m, 4H), 5.47 (dd, J = 13.2, 10.4 Hz, 2H), 5.41 (t, J = 2.9 Hz, 2H), 5.40-5.35 (m, 2H), 5.20-5.16 (m, 1H), 5.11-4.97 (m, 5H), 4.90-4.81 (m, 2H), 3.24 (q, J = 7.3 Hz, 17H), 1.32 (t, J = 7.3 Hz, 25H). ^{13}C NMR (101 MHz; MeOD/$CDCl_3$): δ 131.6, 131.2, 125.26, 125.21, 125.09, 124.93, 124.87, 124.78, 70.25, 70.22, 70.19, 69.95, 69.90, 65.18, 65.11, 65.07, 65.00, 64.85, 64.78, 42.4, 4.2; ^{31}P NMR (162 MHz; MeOD/$CDCl_3$): δ -7.8, -8.9

Compound **PSPSPS ((2a)b)**: Compound **H** (32 mg) was dissolved in 3 mL H_2O. A small scoop of Pd on activated carbon (10%) was added, the mixture was placed under a H_2 atmosphere and stirred for 4 h. The mixture was then purged with N_2 and a drop of NH_4OH was added. The mixture was filtered though celite and evaporated on a rotavap. The residue was dissolved in 1 mL water, loaded on a Vac 6cc 1g tC18 Sep-Pak cartridge (Waters) and eluted with water. The eluted fractions were lyophilized and analyzed by 1H NMR. Yielded 16 mg of **PSPSPS**$\cdot 2Et_3NH^+ \cdot xNH_4^+$. ^1H-NMR (400 MHz; D_2O): δ 4.93-4.78 (m, 3H), 4.55-4.39 (m, 3H), 3.13 (q, J = 7.3 Hz, 14H), 1.21 (t, J = 7.3 Hz, 21H). ^{31}P NMR (162 MHz; D_2O): δ -0.3, -0.7.

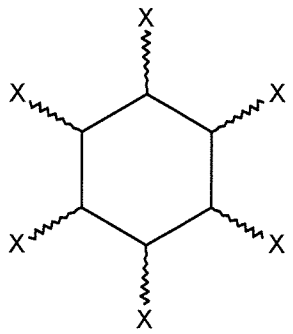
The cleavage induced by compound **PSPSPS ((2a-b)** was determined in the presence of calcium as described in example 2 and the result is shown in Fig. 7. The cleavage induced by this derivative was 50% at a concentration of 20 µM, which is more efficient than IP6 (601 µM). This result shows that the presence of some sulfate groups enhances the activity of the compound in the presence of calcium.

Unless the context clearly requires otherwise, the words "comprise", "comprising" and the like, throughout the description and the claims, are to be construed in an inclusive sense as opposed to an exclusive sense, that is to say, in the sense of "including, but not limited to".

2017202419 12 Apr 2017

Claims

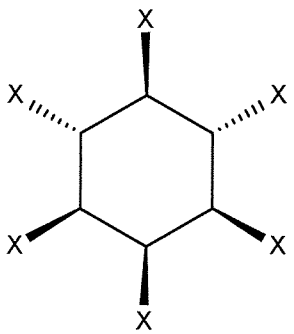
1. A compound described by a general formula (1)



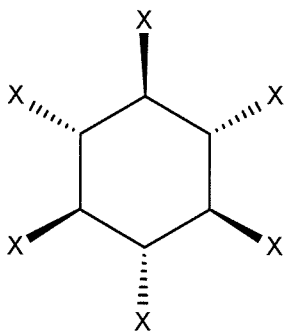
(1)

wherein each X independently is selected from OPO_3^{2-}, $OPSO_2^{2-}$, or OSO_3^-, with the proviso that not all X are OPO_3^{2-} and not all X are OSO_3^-.

2. A compound according to claim 1, wherein the compound is of general formula (1a) or (1b),

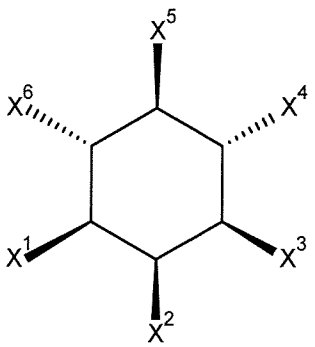


(1a)

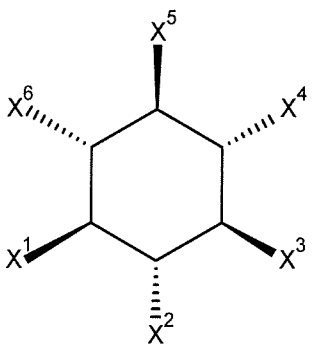


(1b).

3. A compound according to the above claims 1 or 2, of formula (2a) or (2b),



(2a)



(2b),

wherein

a) X^2 is OSO_3^-, and X^1, X^3, X^4, X^5 and X^6 are each independently selected from OPO_3^{2-}, $OPSO_2^{2-}$ or OSO_3^-;

b) X^1, X^3 and X^5 are OPO_3^{2-} and X^2, X^4 and X^6 are OSO_3^-
c) X^1, X^3 and X^5 are OSO_3^- and X^2, X^4 and X^6 are OPO_3^{2-}
d) X^4, X^5 and X^6 are OSO_3^- and X^1, X^2 and X^3 are OPO_3^{2-},
e) X^4, X^5 and X^6 are OPO_3^{2-} and X^1, X^2 and X^3 are OSO_3^-or
f) X^2 and X^5 are OPO_3^{2-} and X^1, X^3, X^4, and X^6 are OSO_3^-,
g) X^2 and X^5 are OSO_3^- and X^1, X^3, X^4, and X^6 are OPO_3^{2-},
h) X^2 and X^3 are OPO_3^{2-} and X^1, X^4, X^5, and X^6 are OSO_3^-, or
i) X^2 and X^3 are OSO_3^- and X^1, X^4, X^5, and X^6 are OPO_3^{2-}.

4. A dosage form, comprising a compound according to any one of claims 1 to 3.

5. The dosage form according to claim 4, further comprising an antibiotic.

6. The dosage form according to claim 5, wherein the antibiotic is metronidazole, vancomycin or fidaxomicin.

7. The dosage form according to any one of claims 4 to 6 formulated as a tablet, capsule, solution, powder or syrup.

8. A method of treating or preventing a C. difficile infection, comprising administering the compound according to any one of claims 1 to 3 or the dosage form according to any one of claims 4 to 7.

9. The use of a compound according to any one of claims 1 to 3 for the manufacture of a medicament for the therapeutic and/or prophylactic treatment of C. difficile infection.

Fig. 1

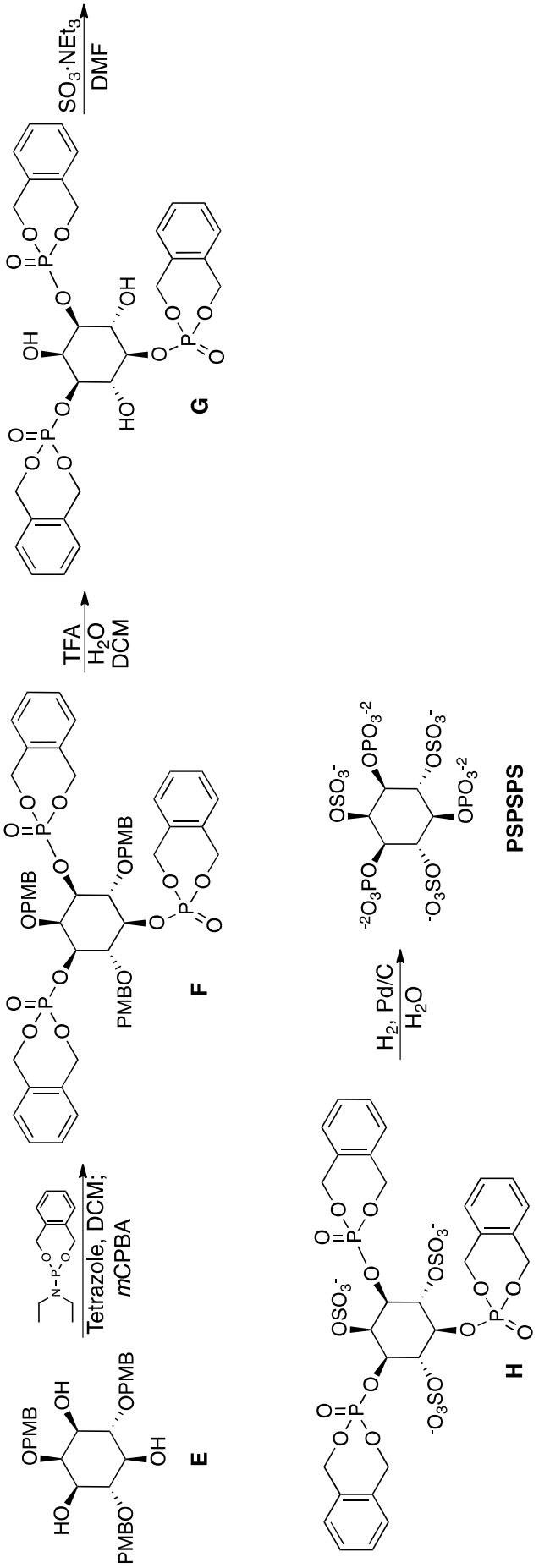
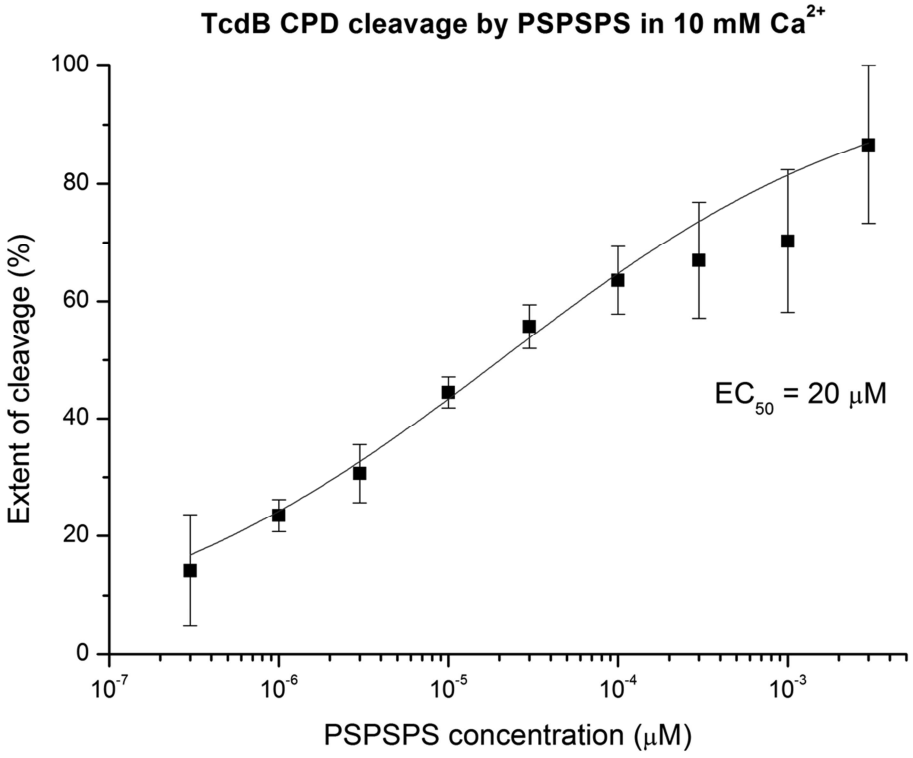

Tetrazole, DCM;
mCPBA
TFA
H2O
DCM
SO3·NEt3
DMF
H2, Pd/C
H2O
E
F
G
H
PSPSPS

Fig. 2


TcdB CPD cleavage by PSPSPS in 10 mM Ca2+
Extent of cleavage (%)
100
80
60
40
20
0
10-7
10-6
10-5
10-4
10-3
PSPSPS concentration (μM)
EC50 = 20 μM