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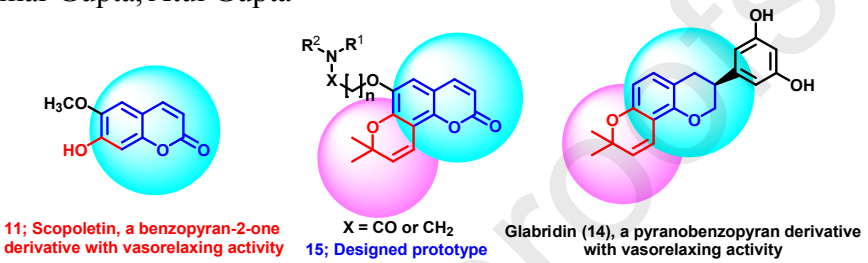

11; Scopoletin, a benzopyran-2-one derivative with vasorelaxing activity

X = CO or CH_2
15; Designed prototype

Glabridin (14), a pyranobenzopyran derivative with vasorelaxing activity

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Synthesis and evaluation of substituted 8,8-dimethyl-8*H*-pyrano[2,3-*f*]chromen-2-one derivatives as vasorelaxing agents

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ABSTRACT

A series of substituted 8,8-dimethyl-8*H*-pyrano[2,3-*f*]chromen-2-ones (chromeno-coumarin hybrids) was synthesized from scopoletin (**11**) as vasorelaxing agents. The synthesized compounds **21a-f, 22, 23a-e** and scopoletin (**11**) were evaluated for vasorelaxation in endothelium intact rat main mesenteric artery (MMA). Compounds **11**, **21a**, **21c-f** and **22** showed significant vasorelaxation in precontracted MMA within the range of EC_{50} value 1.58-5.02 µM. These derivatives presented 29.40-70.89 fold increased sensitivity for experimental tissue compared to scopoletin (**11**), the parent molecule. Among others, **22** was found to be the most active compound which had EC_{50} 1.58µM with 70.89 fold increased sensitivity. The mechanistic evaluation of **22** showed that it exerted vasorelaxation through Ca^{2+}-activated K^+ (BKca) channel and the effect was endothelium-independent.

In drug discovery, oxygen heterocyclic compounds are preferred choice of medicinal chemists over sulfur and nitrogen heterocyclic compounds owing to their relatively less susceptibility for inherent toxicity.[1] Plants have good repository of oxygen heterocycles including furans such as menthofuran (**1**), benzofurans such as machicendiol (**2**), conocarpan (**3**) and Cicerfuran (4), benzopyrans such as daidzein (**5**), genistein (**6**), precocene-I and II (**7** and **8**), glabridin (**9**), osthole (**10**), scopoletin (**11**), and benzoxepins such as ovafolinin A-C (**12-14**) which often have pharmacological activities and serve as templates for novel drugs (Fig. 1).[2-8] Among these, majority of compounds incorporate benzopyran core as an integral part of their structure. These molecules possess benzopyran core mainly in three forms (1) benzopyran (e.g. glabridin), (2) benzopyran-4-one (e.g. flavonoids) and (3) benzopyran-2-one (e.g. coumarins including neoflavones).[3,5,9]

It has been observed that in general, compounds having benzopyran-4-one and benzopyran core presents superior biological activities compared to benzopyran-2-one derivatives possibly due to their structural arrangement which dictates physicochemical properties. Despite this fact, the diverse biological activities such as antioxidant, anticoagulant, antibacterial, antiviral, anticancer, osteoprotective, hepatoprotective, antihypertensive and aromatase inhibitory activities, etc. of benzopyran-2-one derivatives (e.g. **10** and **11**) reveal that benzopyran-2-one core is a valuable pharmacophore for the development of potential bioactive molecules.[8-12] Literature reports suggest that adequate structural changes in benzopyran-2-one core through introduction or transformation of a functional group or core modification leads to its better pharmacological activities.[13,14] In their efforts, You et. al. showed that introduction of an aryl group at C-3 position of osthole (**10**), a benzopyran-2-one derivative, improved its anticancer activity 100 times against estrogen receptor positive breast cancer.[15] Similarly, introduction of an aryl group at C-7 of scopoletin (**11**), another benzopyran-2-one derivative, improved its anticancer activity significantly.[16,17] Besides, functional group manipulations, hybridization of stilbenes such as resveratrol and pterostilbene with benzopyran-2-one derivatives either at C-4 or C-6 position presented superior anticancer activity compared to parent molecules.[18,19] Taken together, positions C-3, C-4, C-6 and C-7 of

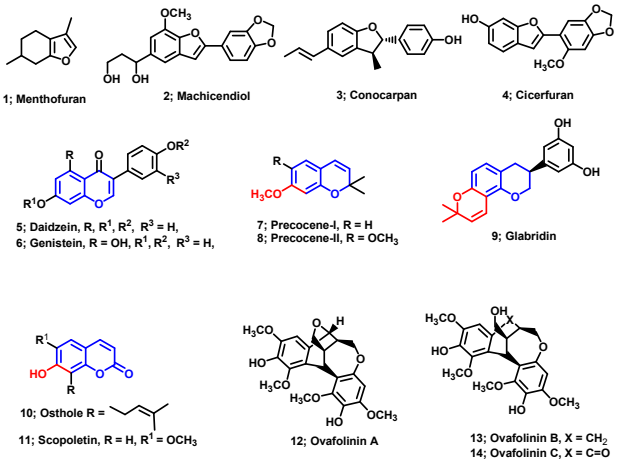

Fig. 1 Structures of naturally occurring oxygen heterocycles (**1-14**) incorporating furan, benzofuran, pyran, benzopyran, benzoxepins core.

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benz…tion modifications in order to improve its pharmacological activities.[25]

Considering above observations and medicinal importance of benzopyran-2-one core, in this study, we undertook structural hybridization of scopoletin (**11**), a benzopyran-2-one derivative, with benzopyran (chromene) nucleus as present in precocene-I and II (**6a** and **b**), to yield substituted chromeno-coumarin hybrids (**15**) in anticipation to have effective vasorelaxant agents (Fig. 2). It is known that benzopyran-2-one core is responsible for vasorelaxing activity of many active molecules.[21-24] Moreover, pyranobenzopyran core present in glabridin (**14**), a known vasorelaxing agent, is an important core for biological activity of **14**.[25] It was assumed that these 8,8-dimethyl-8*H*-pyrano[2,3-*f*]chromen-2-ones (chromeno-coumarin hybrids, **15**) may have improved physicochemical properties required for better interaction with biological targets as compared to scopoletin (**11**) which is known for its moderate vasorelaxant activity rather at high concentration.[21] The results are reported in this communication.

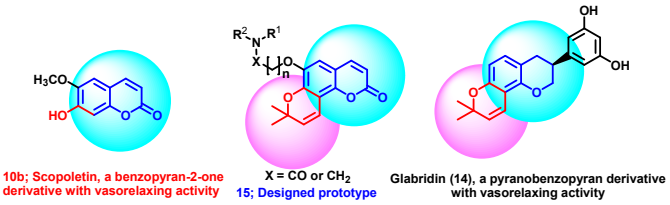
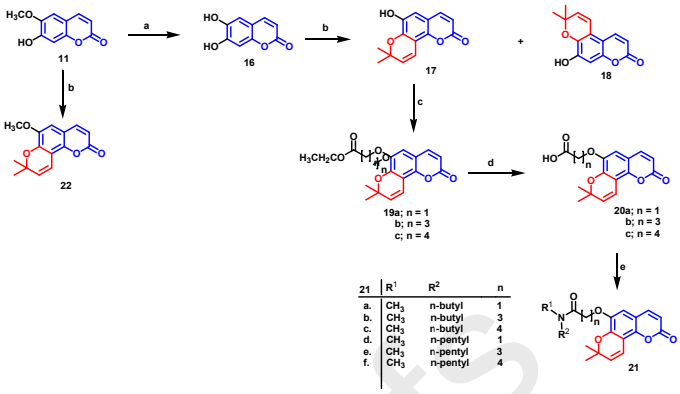


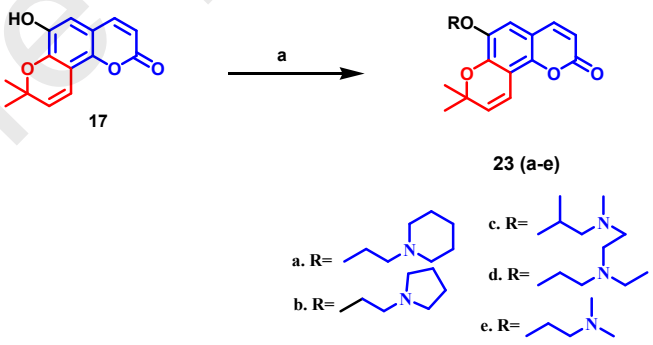
Fig. 2 Structures of benzopyran-2-one (scopoletin, **11**) and pyranobenzopyran (glabridin, **8**) based vasorelaxing agents and designed prototype.

The designed chromeno-coumarin hybrids (**15**) were synthesized using efficient chemistry (Scheme-1). For synthesis of target compounds, scopoletin (**11**) was required as starting material which was isolated from stem part of the plant *Artemisia annua* L. cv CIM sanjeevnai collected from CIMAP research form. The stem of this plant is a major bio-waste of the *Artemisia* research and was taken for its value addition in this study. The dried and powdered plant material was extracted with hot methanol. The obtained crude material was subjected to column chromatography in order to purify scopoletin (**11**). Subsequently, scopoletin (**11**) was demethylated using pyridinium hydrochloride at 150°C which yielded 6,7-dihydroxybenzopyran-2-one (**16**) in 70% yield. O-alkylation followed by cyclization reaction was performed on **16** using 1,1-diethoxy-3-methyl-2-butene in dry xylene at 160°C to yield 6-hydroxy-8,8-dimethyl-8*H*-pyrano[2,3-*f*]chromen-2-one (**17**) along with 7-hydroxy-8,8-dimethyl-8*H*-pyrano[2,3-*f*]chromen-2-one (**18**) in 77 and 11 % yields respectively. Compound **17** was reacted with alkyl bromoesters of variable lengths in presence of anhydrous K_2CO_3 in dry acetone to yield **19** in 81-92% yields. The ester derivatives (**19**) were hydrolyzed to the corresponding acid derivatives (**20**) in 70-82 yields using 10% aqueous NaOH in MeOH at room temperature. Finally, **20** was transformed into target amide derivatives (**21**) using different alkylamines in presence of EDC, HOBt in DCM at room temperature in 50-71 % yields. In 2002, Rokotoarison et. al. reported vasorelaxing activity of crude hydroalcoholic extract of trunk bark of *Cedrelopsis grevie* Baill. (Meliaceace) containing scopoletin derivatives, [6, 7-dimethoxy benzopyran-2-one and 8-(3-methylprop-2-enyl)- benzopyran-2-one] and chromeno-coumarin hybrids [6-hydroxy 8,8-dimethyl-8*H*-pyrano[2,3-*f*]chromen-2-one (norbraylin, **17**), 7-hydroxy-8,8-dimethyl-8*H*-pyrano[2,3-*f*]chromen-2-one (cedrecoumarin A, **18**) and 7-methoxy- 8,8-dimethyl-8*H*-pyrano[2,3-*f*]chromen-2-one (braylin, **22**)].[26] Despite of their interest, these authors could not study vasorelaxaing effect of individual constituents except one of the scopoletin derivative i.e. 6, 7-dimethoxy benzopyran-2-one, due to their insufficient quantities. Encouraged with the observed activity of extract and 6, 7-dimethoxy benzopyran-2-one, we were interested to study **22** in detail. Accordingly, we synthesized 7-methoxy-8,8-dimethyl-8*H*-pyrano[2,3-*f*]chromen-2-one (braylin, **22**). Compound reaction on **11** using 1,1-diethoxy-3-methyl-2-butene in dry xylene at 160°C in 75% yield.



Scheme 1. Reagents and reaction conditions: (a) pyridinium hydrochloride at 150°C (b) 1,1-diethoxy-3-methyl-2-butene, 3-picoline in dry *p*-xylene at 160°C (c) alkyl bromoester, anhy K_2CO_3, dry acetone, reflux (d) 5% aq.NaOH, MeOH at room temperature (e) EDC, HOBt, DMAP, DCM at room temperature.

Furthermore, 6-hydroxy-8,8-dimethyl-8*H*-pyrano[2,3-*f*]chromen-2-one (**17**) was modified to its alkylamine substituted derivatives (**23**) through the reaction of **17** with substituted alkylamine hydrochlorides in presence of anhydrous K_2CO_3 in dry acetone (Scheme-2). Thus, alkylation reaction on **17** yielded corresponding alkylamine substituted derivatives **23** in 51-76% yields



Scheme 2; Reaction conditions and reagents: (a) alkylaminoethyl chloride.hydrochloride, anhy K_2CO_3, dry acetone, 80°C.

The synthesized compounds were analyzed with the help of ^1H, ^{13}C NMR and mass spectrometry and compounds with minimum 97 % purity were evaluated for their vasorelaxant activity in isolated rat main mesenteric artery (MMA), one of the main circulatory bed, using ex-vivo model [see supplementary information].

The vasorelaxant potential of target molecules was studied in endothelium intact MMA precontracted with U46619 (100nM). The endothelium integrity of isolated arterial rings was confirmed using acetylcholine-induced relaxation. Initially, **11**, **21a-f**, **22** and **23a-e** were evaluated for vasorelaxation in MMA at 30µM except **11** which was evaluated at 1000 µM concentration, to have a general idea about vasorelaxant activity of compounds. Results showed that **11**, **21a, 21c-f** and **22** showed 63.57-96.70 % relaxation (E_{max}) in intact MMA precontracted with U46619 (100nM) whereas compounds **21b** and **23a-e** showed less than 10% relaxation and were considered inactive (Table 1). Compounds **21a, 21c-f** and **22** were further evaluated for their efficacy in a concentration-dependent manner.

The vasorelaxation by each compound was normalized to calculate percentage of the maximal vasorelaxation obtained in the normal control group. The concentration of the test compound causing half-maximal relaxation (EC_{50}) and maximal relaxation (E_{max}) were calculated by fitting the original concentration-response curves with nonlinear regression equation. The activity of compounds is

repr... this EC_{50} (Table 1). It was observed that **21a, 21c-f** and **22** presented significant vasorelaxation in MMA precontracted with U46619 (100nM) within the range of EC_{50} value 1.58-5.02 µM and pD2 value 5.299±0.09-5.802±0.08 (Table 1). Accordingly, the increased sensitivity of experimental tissue for **21a, 21c-f** and **22** was observed from 22.31-70.89 fold compared to scopoletin (**11**), the parent experiment, sodium nitroprusside was used as positive control and presented E_{max} 91.39 at 1µM (Table 1). Among others, **22** was found to be the most active compound [EC_{50} 1.58µM with 70.89 fold increased sensitivity (E_{max} 94.01 for **22** and 89.23% for **11** respectively) Table 1] and was selected for further characterization.

Table 1. Vasorelaxation potential of **11, 21a-f, 22** and **23a-e** in rat main mesenteric artery.

S. No.	Compound	E_{max} (at 30 µM)#	pD2 (-LogM EC_{50})	EC_{50} in µM	Fold increase in sensitivity
1	Scopoletin (**11**)*	89.23	3.95±0.09	112	1.00
2.	**21a**	65.67	5.575±0.09	2.66	42.11
3	**21b**	<10	ND	ND	ND
4	**21c**	63.57	5.674±0.08	2.12	52.83
5	**21d**	94.05	5.419±0.07	3.81	29.40
6	**21e**	95.06	5.491±0.10	3.23	34.67
7	**21f**	83.12	5.299±0.09	5.02	22.31
8	**22**	96.70	5.802±0.08	1.58	70.89
9	**23a**	<10	ND	ND	ND
10	**23b**	<10	ND	ND	ND
11	**23c**	<10	ND	ND	ND
12	**23d**	<10	ND	ND	ND
13	**23e**	<10	ND	ND	ND
14	Sodium Nitroprusside≠	91.39	8.524±0.11	0.003	Standard

*E_{max} of scopoletin (**11**) is measured at 1000 µM. ≠ E_{max} of Sodium Nitroprusside is measured at 1000 µM. #E_{max} is the maximal relaxation in terms of percent at 30 µM in comparison to scopoletin, **11**). ND = not determined.

In order to elucidate the role of endothelium integrity on vasorelaxation, **22** was evaluated on MMA both in presence and absence of endothelium. Result showed that **22** relaxed MMA both in presence and absence of endothelium. The concentration-dependent relaxation response of **22** in endothelium intact and denuded showed no statistical difference with E_{max} 94.87% for endothelium intact and 93.69% for endothelium-denuded aortic rings respectively (Fig. 3). This observation showed that relaxation response of **22** was endothelium-independent.

Further, to elucidate the role of potassium channels, relaxation efficacy of **22** was assessed in MMA pre-contracted with high potassium depolarizing solution [80mM KCl containing Modified Krebs-Henseleit Solution (MKHS)] which prevents opening of potassium channels through increase in external potassium ion concentrations. The relaxation response by **22** was found to be significantly blocked with E_{max} 94.01 % for control and 53.34 % for 80mM KCl containing MKHS respectively. Furthermore, the contribution of subtypes of potassium channels in the relaxation response of **22** was studied by incubating MMA with Tetraethylammonium (TEA, Ca^{2+} -activated K^+ channel blocker; BK_{ca} channel) for 20min. It was found that pre-incubation of the arterial rings with TEA (1mM) for 20 min significantly blocked the relaxation response to **22** with E_{max} 54.13±5.58 % compared to control (E_{max} 94.01±1.49 %) (Fig. 4).

The observed vasorelaxation in MMA by **22** was validated through *in-silico* docking study on BKca channel target (PDB ID: 3NAF) using Auto Dock Vina v1.1.2 software. In this experiment, tetraethylammonium (TEA) was used as control molecule. The results showed that **22** accommodated itself well within the binding pocket of target protein with binding energy -7.5 $kcal.mol^{-1}$ and formed two hydrogen bonds with ARG-514 residue through C=O and one of the O-atom (form pyranone ring) whereas TEA interacted within target with binding energy -3.7 $kcal.mol^{-1}$. It was seen that binding pocket of TEA and **22** within the target was not same. The results are presented in Fig. 5. The *in-silico* results indicated that **22** had ability to target BKca channel for its vasorelaxation activity.

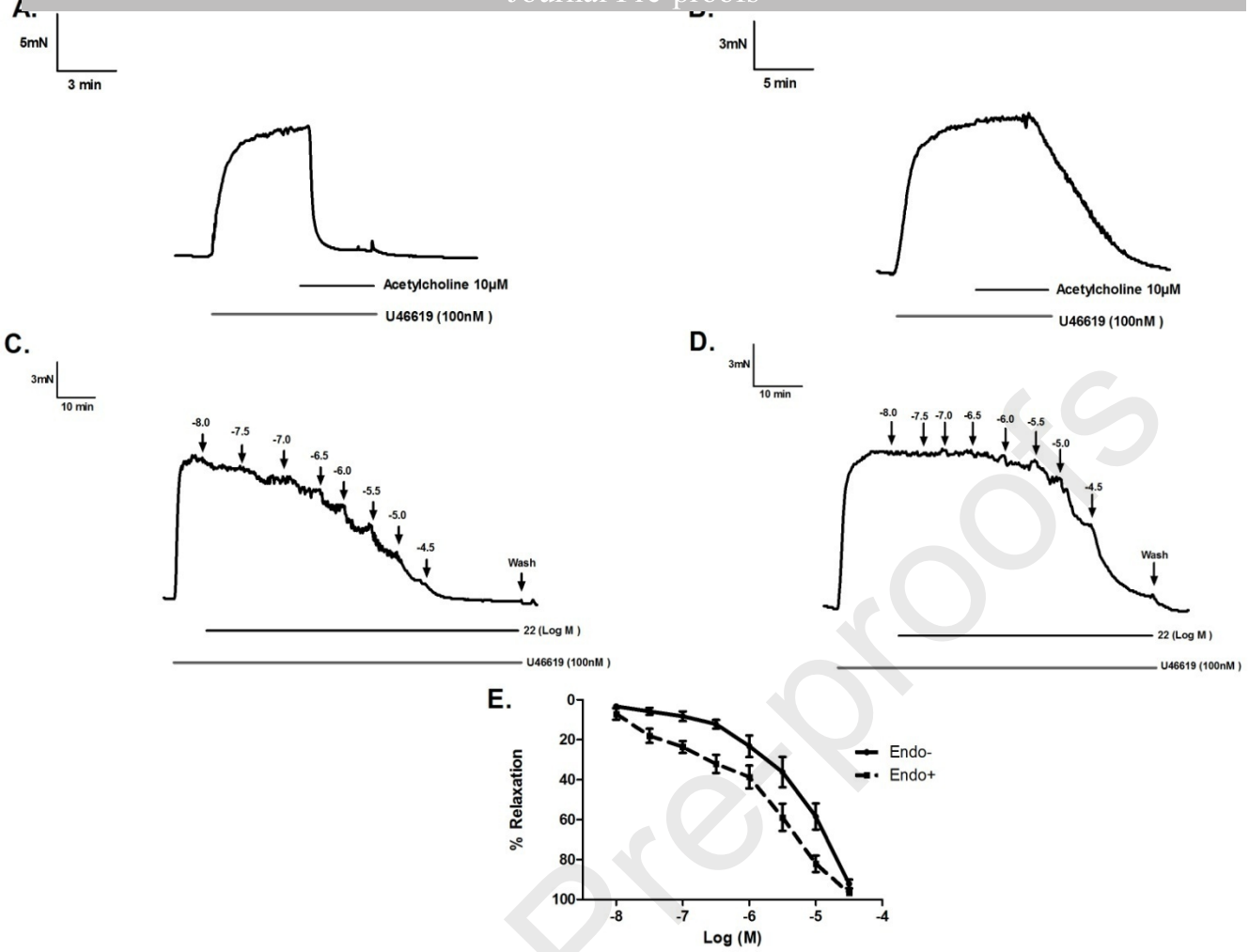


Fig 3. Traces showing acetylcholine (10 μM) induced relaxation in endothelium intact (A), endothelium-denuded (B), isolated rat mesenteric arterial rings preconstricted with U46619 (100 nM). Raw traces shows **22** induced concentration-dependent relaxation in endothelium intact (C), endothelium-denuded (D), isolated rat mesenteric arterial rings preconstricted with U46619 (100 nM). (E) graph shows the sigmoidal concentration-response curve obtained in C and D. Relaxation is expressed as the Mean ± S.E.M. percentage reversal of U46619-induced contraction.

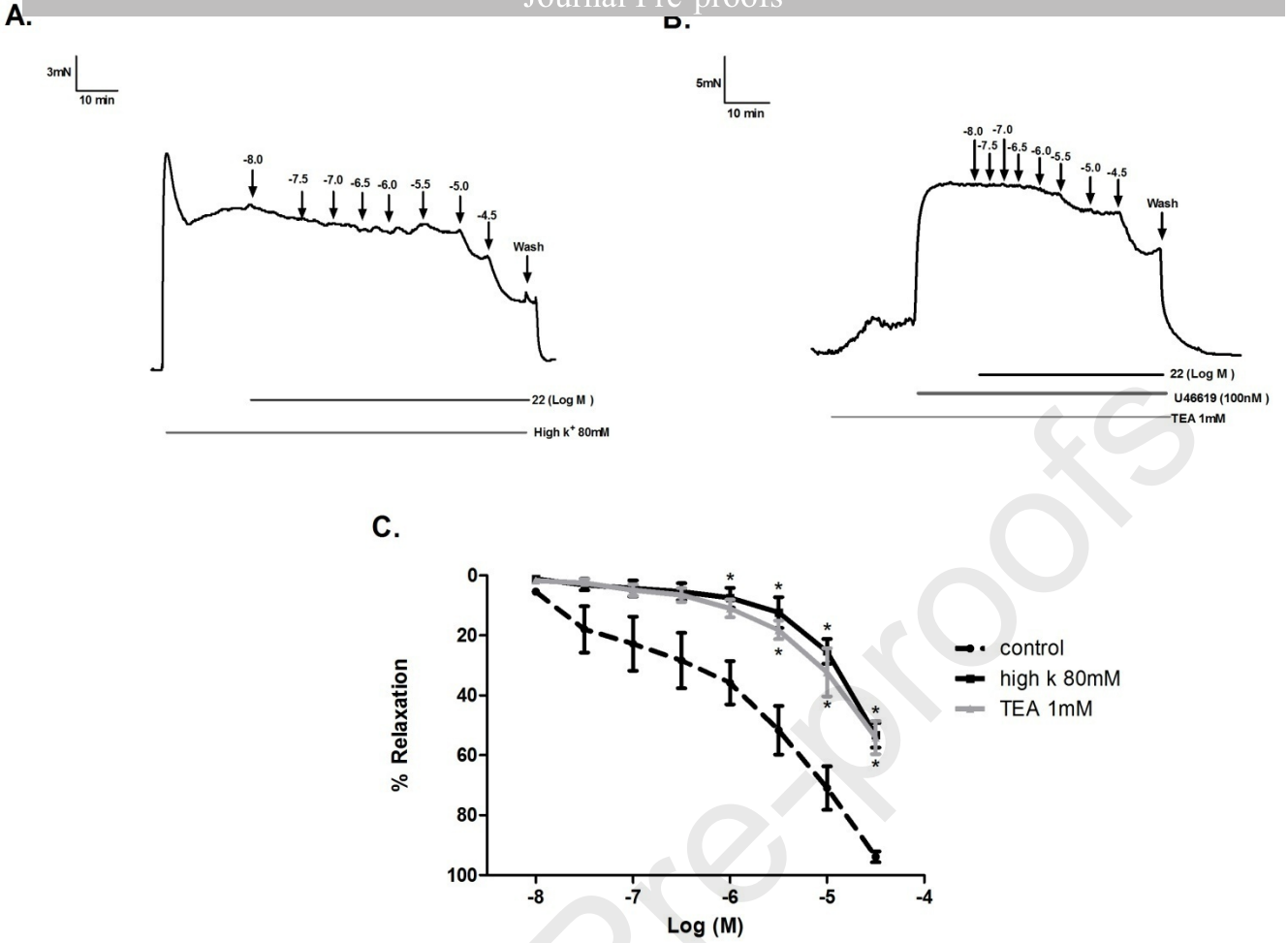
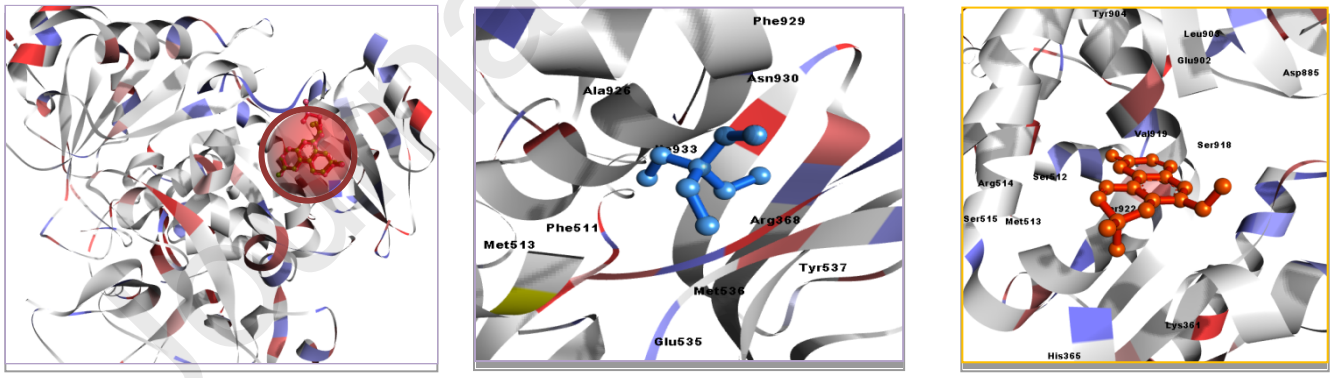


Fig 4. Traces showing **22** induced concentration-dependent relaxation in rat mesenteric arterial rings preconstricted with 80 mM High K^+ containing MKHS (A) and pre-incubated with potassium channel blocker; Tetraethyl ammonium (TEA, 1 mM), for 20 min and preconstricted with U46619 (100 nM) Tracing (B). Graph shows the sigmoidal concentration-response curve (C). Relaxation is expressed as the Mean ± S.E.M. percentage reversal of contraction.



(A) (B) (C)

Fig 5. (A). Structural crystallographic model of human BKca channel (PDB ID: **3NAF**) showing binding site in orange color sphere. **(B).**Docking pose conformation of tetraethylammonium with BKca channel (C) Represent the docking pose of **22** with BKca channel.

These findings indicated that vasorelaxation effect of **22** was mediated through Ca^{2+}-activated K^+ (BKca) channel. It has been reported that Voltage-Dependent Calcium Channel (VDCC) and store-operated calcium channels are putative targets of coumarins.[27] Further, in myocardial infarction, enhanced platelet aggregation followed by thrombus formation is a causative factor which is modulated by calcium mobilization, cox-1 and thromboxane A_2 receptors etc. It is reported that 4-methylcoumarin derivatives are reported to inhibit platelet aggregation induced by U-46619 through antagonistic action at thromboxane-A_2 receptor.[28] Considering, antagonistic action of coumarins at thromboxane-A_2 receptor and good platelet aggregation activity of **22**, the antagonistic action of **22** at thromboxane-A_2 receptor may be another possible mode for its vasorelaxation effect.

The above results showed that structural modification of scopoletin (**11**) to yield substituted 8,8-dimethyl-8*H*-pyrano[2,3-*f*]chromen-2-ones (chromeno-coumarin hybrids, **15**) improved its vasorelaxation efficacy. Compounds having amide group at position C-6 of 8,8-dimethyl-8*H*-pyrano[2,3-*f*]chromen-2-one presented better activity with enhanced sensitivity towards MMA compared to scopoletin

(**11**) group at C-6 presented even more pronounced activity. It was assumed that long-chain modification at this position is not favorable perhaps due to its bulky nature. In a similar study, it was observed that 4-hydroxymethylcoumarins bearing 2-oxopropyl group at its C-7 and C-8 positions were devoid of vasorelaxant effect, however, transformation of 2-oxopropyl group present at C-7, to a methyl group (a small lipophilic group analogous to methoxy group of **22**) bearing furan ring fused coumarin (through C-6 and C-7 positions) induced vasorelaxant effect.[29] This observation indicated the importance of a small lipophilic group at C-6 and medium sized at C-8 in coumarins for their vasorelaxant effect. Similarly, vasorelaxant activity of umbelliferone-chalcone hybrids which had lipophilic cinnamoyl group at C-8 also validated this fact.[30] The observed activity of compounds along with the these literature reports indicated that structurally, active compound required a small lipophilic group (of two atoms) at C-6 and medium sized at C-8, which may or may not be connected to C-7 through an O-atom. As anticipated, the modification of scopoletin (**11**) to chromeno-coumarin hybrids significantly improved its vasorelaxation efficacy; however, a proper structure-activity relationship could not be established in this series.

In conclusion, a series of 8,8-dimethyl-8*H*-pyrano[2,3-*f*]chromen-2-ones (chromeno-coumarin hybrids) has been synthesized efficiently through structural modification of scopoletin (**11**) and characterized as vasorelaxing agent with enhanced vasorelaxation and sensitivity in rat main mesenteric artery (MMA) compared to scopoletin (**11**), the parent molecule. Amongst other, **22** showed significant vasorelaxation through Ca^{2+} activated K^+ (BKca) channel.

Together with the observed activity, it is assumed that 8*H*-pyrano[2,3-*f*]chromen-2-one based molecules such as **22**, could be developed as novel vasorelaxing agents after further structural refinements and detailed biological characterizations.

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References and notes

1. Zhao L, Yuan X, Wang J, Feng Y, Ji F, Li Z, Bian J. A review on flavones targeting serine/threonine protein kinases for potential anticancer drugs. *Bioorg. Med. Chem*., 2019, *27*, 677-685. https://doi.org/10.1016/j.bmc.2019.01.027; (b) Kumar D, Sharma P, Singh H, Nepali K, Gupta G. K, Jain S. K, Ntie-Kang F. The value of pyrans as anticancer scaffolds in medicinal chemistry. *RSC Adv.*, 2017, *7*, 36977-36999. https://doi.org/10.1039/C7RA05441F.
2. Dawood KM. Benzofuran derivatives: a patent review. *Expert Opin. Ther. Patents,* 2013, *23*, 1133-1156.
3. (a) Ren W, Qiao Z, Wang H, Zhu L, Zhang L. Flavonoids: Promising anticancer agents. *Med. Res. Rev.,* 2003, *23*, 519-534. https://doi.org/10.1002/med.10033; (b) Raffa D, Maggio B, Raimondi MV, Plescia F, Daidone G. Recent discoveries of anticancer flavonoids. *Eur. J. Med. Chem*., 2017, *142*, 213. https://doi.org/10.1016/j.ejmech.2017.07.034.
4. Kamboj A, Saluja AK. *Ageratum conyzoides* L.: A review on its phytochemical and pharmacological profile. *Int. J. Greeen. Pharma*, 2008, *2,* 59-68.
5. Simmler C, Pauli GF, Chen AN. Phytochemistry and biological properties of glabridin. *Fitoterapia*, 2013, 90, 160-184. https://doi.org/10.1016/j.fitote.2013.07.003.
6. Yao L, Lu P, Li Y, Yang L, Feng H, Huang Y, Zhang D, Chen J, Zhu D. Osthole relaxes pulmonary arteries through endothelial phosphatidylinositol 3-kinase/Akt-eNOS-NO signaling pathway in rats. *Eur. J. Pharmacol.*, 2013, *699*, 23-32. https://doi.org/10.1016/j.ejphar.2012.11.056.
7. Lee SH, Ding Y, Yan XT, Kim YH, Jang HD. Scopoletin and Scopolin Isolated from *Artemisia iwayomogi* Suppress Differentiation of Osteoclastic Macrophage RAW 264.7 Cells by Scavenging Reactive Oxygen Species. *J. Nat. Prod.,* 2012, *76*, 615-620. https://doi.org/10.1021/np300824h.
8. [illegible] H. Ovalonimins A-E, Five New Lignans from Lyonia ovalifolia, *Chem. Pharm. Bull.*, 2010, *58*, 191-194.
9. Kontogiorgis C, Detsi A, Hadjipavlou-Litina D. Coumarin-based drugs: a patent review (2008-present). *Expert Opin. Ther. Pat.,* 2012, *22*, 437-454. https://doi.org/10.1517/13543776.2012.678835.
10. Gnonlonfin GJB, Sanni A, Brimer L. Review Scopoletin-A Coumarin Phytoalexin with Medicinal Properties. *Crit. Rev. Plant Sci.*, 2012, *31*, 47-56. https://doi.org/10.1080/07352689.2011.616039.
11. Singh H, Singh JV, Bhagat K, Gulati HK, Sanduja M, N. K. N. Kinarivala NKN, Sharma S. Rational Approaches, Design Strategies, Structure Activity Relationship and Mechanistic Insights for Therapeutic Coumarin Hybrids. *Bioorg. Med. Chem. Lett.*, 2019, *27*, 3477-3510. https://doi.org/10.1016/j.bmc.2019.06.033.
12. (a)Jameel E, Umar T, Kumar J, Hoda N. Coumarin: A Privileged Scaffold for the Design and Development of Antineurodegenerative Agents. *Chem. Biol. Drug Des*. 2016, *87*, 21-38. https://doi.org/10.1111/cbdd.12629. (b) Grover J, Jachak SM. Coumarins as privileged scaffold for anti-inflammatory drug development. *RSC Adv.,* 2015, *5*, 38892-38905. https://doi.org/10.1039/C5RA05643H. (c) Peng XM., Damu GLV, Zhou CH. Current developments of coumarin compounds in medicinal chemistry. *Curr. Pharm. Design,* 2013, *19*, 3884-3930. DOI:10.2174/1381612811319210013.
13. Emamia S, Dadashpour S. Current developments of coumarin-based anti-cancer agents in medicinal chemistry. *Eur. J. Med. Chem*., 2015, *102*, 611-630. https://doi.org/10.1016/j.ejmech.2015.08.033.
14. Thakur A, Singla R, Jaitak V. Coumarins as anticancer agents: A review on synthetic strategies, mechanism of action and SAR studies, *Eur. J. Med. Chem*., 2015, *101*, 476-495. https://doi.org/10.1016/j.ejmech.2015.07.010.
15. You L, An R, Wang X, Li Y. Discovery of novel osthole derivatives as potential anti-breast cancer treatment. *Bioorg. Med. Chem. Lett.*, 2010, *20*, 7426-7428. https://doi.org/10.1016/j.bmcl.2010.10.027.
16. Cai X, Yang J, Zhou J, Lu W, Hu C, Gu Z, Huo J, Wang X, Cao P. Synthesis and biological evaluation of scopoletin derivatives. *Bioorg. Med. Chem.*, 2013, *21*, 84-92. https://doi.org/10.1016/j.bmc.2012.10.059.
17. Yang J, Guo-Yun L, Dai F, Cao XY, Kang YF, Hu LM., Tang JJ, Li XZ, Li Y, Jin XL, Zhou B, Synthesis and biological evaluation of hydroxylated 3-phenylcoumarins as antioxidants and antiproliferative agents. *Bioorg. Med. Chem. Lett.*, 2011, *21*, 6420-6425. https://doi.org/10.1016/j.bmcl.2011.08.090.
18. W. Shen, J. Mao, J. Sun, M. Sun, C. Zhang, Synthesis and biological evaluation of resveratrol–coumarin hybrid compounds as potential antitumor agents. *Med. Chem. Res*., 2013, *22*, 1630-1640. https://doi.org/10.1007/s00044-012-0159-y.
19. Belluti F, Fontana G, Bo LD, Carenini N, Giommarelli C, Zunino F, Design, synthesis and anticancer activities of stilbene-coumarin hybrid compounds: Identification of novel proapoptotic agents, *Bioorg. Med. Chem.*, 2010, *18*, 3543-3550. https://doi.org/10.1016/j.bmc.2010.03.069.
20. Dandriyal J, Singla R, Kumar M, Jaitak V, Recent developments of C-4 substituted coumarin derivatives as anticancer agents, *Eur. J. Med. Chem*., 2016, *119*, 141-168. https://doi.org/10.1016/j.ejmech.2016.03.087.
21. Kwon EK, Jin SS, Choi MH, Hwang KT, Shim JC, Hwang IT, Han JH, Mechanism of relaxation of rat aorta by scopoletin; an active constitutent of Artemisia capillaris. *Kor. J. Ori. Med. Physiol. Pathol.,* 2002, *16,* 389-396.
22. Vilar S, Quezada E, Santana L, Uriarte E, Yanez M, Fraiz N, Alcaide C, Cano E, Orallo F, Design, synthesis, and vasorelaxant and platelet antiaggregatory activities of coumarin–resveratrol hybrids. *Bioorg. Med. Chem. Lett.*, 2006, *16*, 257-261. https://doi.org/10.1016/j.bmcl.2005.10.013.
23. Wang C, Wang T, Huang L, Lu W, Zhang J, He H, Synthesis and fluorescent study of 5-phenyl furocoumarin derivatives as vasodilatory agents. *Bioorg. Med. Chem. Lett.*, 2016, *26*, 640-644. https://doi.org/10.1016/j.bmcl.2015.11.056.
24. Wang T, Wang C, Zhou N, Pan X, He H, Synthesis and vasorelaxation evaluation of novel biphenyl-furocoumarin derivatives. *Med. Chem. Res*., 2015, *24*, 2417-2431. https://doi.org/10.1007/s00044-014-1303-7.

Aaronson PI, Glabridin-induced vasorelaxation: Evidence for a role of BKCa channels and cyclic GMP. *Life Science*, 2016, *165*, 26-34. https://doi.org/10.1016/j.lfs.2016.09.018.

26. Rakotoarison O, Rabenau I, Lobstein A,. Um BH, Schott C, Anton R, Randriantosa A, Andriantsitohaina R, Vasorelaxing Properties and Bio-Guided Fractionation of *Cedrelopsis grevei.Planta Med.,* 2003*, 69*, 179-181. https://doi.org/10.1055/s-2003-37717.
27. Najmanová I, Doseděl M, Hrdina R, Anzenbacher P, Filipsky T, Říha M, Mladěnka P, Cardiovascular effects of coumarins besides their antioxidant activity. *Curr. Top. Med. Chem*., 2015*, 15,* 830-849. https://doi.org/10.2174/1568026615666150220112437.
28. Macáková K , Reháková Z,Mladenka P,Karlícková J, Filipský T, Ríha M, Prasad AK, Parmar VS, Jahodár L, Pávek P, Hrdina R, Saso L. In vitro platelet antiaggregatory properties of 4-methylcoumarins. *Biochimie*, 2012, *94*, 268-2686. http://dx.doi.org/10.1016/j.biochi.2012.09.006.
29. Campos-Toimil M, Orallo F, Santana L. Uriarte E. Synthesis and Vasorelaxant Activity of New Coumarin and Furocoumarin Derivatives. *Bioorg. Med. Chem. Lett.,* 2002, *12*, 783-786.
30. Amin KM, Awadalla FM, Eissa AAM, Abou-Seri SM, Hassan GS. Design, synthesis and vasorelaxant evaluation of novel coumarins-pyrimidine hybrids. *Bioorg. Med. Chem*., 2011, *19*, 6087-6097. https://doi.org/10.1016/j.bmc.2011.08.037.

Graphical Abstract

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Synthesis and evaluation of substituted 8,8-dimethyl-8*H*-pyrano[2,3-*f*]chromen-2-one derivatives as vasorelaxing agents

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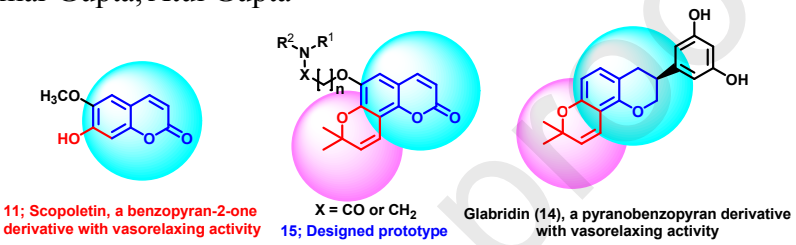
Sarita Singh, Karishma Agarwal, Hina Iqbal, Pankaj Yadav, Deepika Yadav, Debabrata Chanda,* Sudeep Tandon, Feroz Khan, Anil Kumar Gupta, Atul Gupta*



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Date: October 12, 2019

To,
The Editor,

Bioorganic and Medicinal Chemistry Letters

Subject: Submission of revised manuscript (BMCL-D-00909) to Bioorganic and Medicinal Chemistry Letters for publication

Dear Sir/Madam,

Please find enclosed our revised manuscript entitled **"Synthesis and evaluation of substituted 8,8-dimethyl-8*H*-pyrano[2,3-*f*]chromen-2-one derivatives as vasorelaxing agents"** for publication in "Bioorganic and Medicinal Chemistry Letters."

This study reports design and synthesis substituted 8,8-dimethyl-8*H*-pyrano[2,3-*f*]chromen-2-ones (chromeno-coumarin hybrids) from scopoletin (**11**) which were evaluated for their vasorelaxation in endothelium intact rat Main Mesenteric Artery (MMA).

Compounds **11**, **21a**, **21c-f** and **22** showed significant vasorelaxation in precontracted MMA within the range of EC_{50} value 1.58-5.02 μM with 29.40-70.89 fold increased sensitivity for experimental tissue compared to scopoletin (**11**), the parent molecule.

Among others, **22** was found to be the most active compound which had EC_{50} 1.58μM and pD2 5.802±0.08 istic evaluation of **22** showed that it exerted vasorelaxation through Ca^{2+}-activated K^+ (BKca) channel and the effect was endothelium independent.

Thanking you in anticipation.

Best regards,
Dr. Atul Gupta,
Scientist
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